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Report on the 2012 Proficiency Test of the European Union Reference Laboratory for Mycotoxins, for the Network of National Reference Laboratories

Determination of DON, ZON, T-2 and HT-2 in Cereals

Zoltan Kunsagi
Katrien Bouten
Andreas Breidbach
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Joerg Stroka

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Institute for Reference Materials and Measurements

Contact information
Joerg Stroka
Address: Joint Research Centre, Retieseweg 111, B-2440, Belgium
E-mail: joerg.stroka@ec.europa.eu
Tel.: +32 1457 1229
Fax: +32 1457 1783

http://irmm.jrc.ec.europa.eu/
http://www.jrc.ec.europa.eu/

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Project ID: MYCO-FUSAR-07
PT coordinator: Zoltan Kunsagi

September 2012

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1. Summary

The Institute for Reference Materials and Measurements (IRMM) of the Joint Research Centre (JRC), a Directorate-General of the European Commission, operates the European Union Reference Laboratory (EU-RL) for Mycotoxins. One of its core tasks is to organise interlaboratory comparisons (ILCs) among appointed National Reference Laboratories (NRLs).

This report presents the results of the ILC of the EU-RL for Mycotoxins which focused on the determination of deoxynivalenol (DON), zearalenone (ZON), T-2 and HT-2 in cereal samples.

The two test items were naturally contaminated cereal-based animal feed. The two materials were procured by the IRMM and dispatched to the participants in May 2012. Each participant received two sachets containing approximately 100 g of test material each.

Thirty-five participants from 27 countries registered for the exercise. Thirty four (Sample A) and 34 (Sample B) sets of results were reported for DON, 33 & 32 for ZON, 32 & 28 for T-2 and 30 & 28 for HT-2.

The assigned values, established by exact-matching double isotope dilution mass spectrometry", were 605 µg/kg (Sample A) and 282 µg/kg (Sample B) for DON, and 445 and 28 µg/kg for ZON. The uncertainties of the respective assigned values were 49 and 26 µg/kg, and 16 and 4 µg/kg.

Participants were invited to report the uncertainty of their measurements. This was done by the majority of laboratories.

Laboratory results for DON and ZON were rated with z-scores and zeta-scores in accordance with ISO 13528 and the International Harmonized Protocol for the Proficiency Testing of Analytical Chemistry Laboratories.

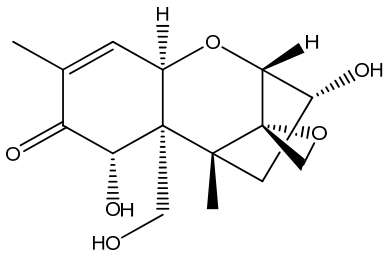
Only z-scores for DON and ZON were used for an evaluation of an underperformance. In total about 95 % of the attributed z-scores were below an absolute value of two for these two mycotoxins, which indicated that most of the participants performed satisfactory or better.

Due to lack of legislative limits and inconclusive data on the assigned values neither z-scores nor zeta-scores were calculated at the moment for T-2 and HT-2.

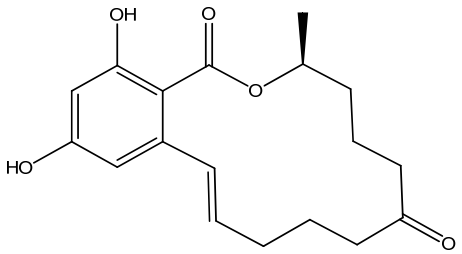
2. Introduction

Figure 1: Chemical structures of the analytes in the proficiency test

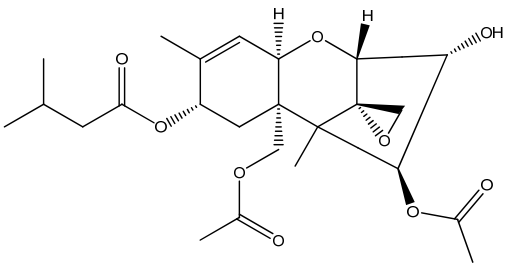
a) DON



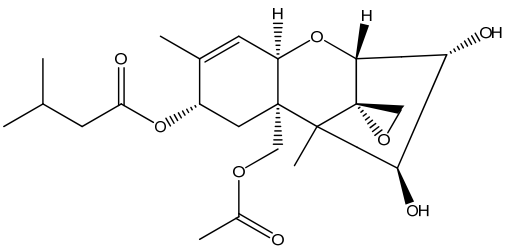
b) ZON



c) T-2



d) HT-2



Fusarium fungi species produce a heterogeneous variety of mycotoxins such as trichothecenes and myco-oestrogens.

The most abundant trichothecenes are deoxynivalenol (DON, vomitoxin, type B) [**Figure 1a**], produced by *F. graminearum* and *F. culmorum*, T-2-toxin and HT-2-toxin (T-2, HT-2, type A) [**Figure 1c-d**], produced by *F. poae*, *F. langsethiae* and *F. sporotrichioides*. These are mainly contaminating cereals like wheat, barley and maize used as food and feed. T-2 can be metabolised into HT-2. Emesis, reduced weight gain and other gastrointestinal disorders are the most sensitive functional manifestations of the type B trichothecenes, while immunotoxicity, cytotoxicity and neurotoxicity are caused by the type A trichothecenes [**1**], [**2**].

The structure of myco-oestrogens (zearalenone and derivatives) resembles oestradiol as it has high oestrogenic activity causing hyperoestrogenism in animals and humans. An oestrogenic response is induced by several organisms, resulting in common symptoms as infertility, vulval oedema and testicular atrophy. Zearalenone (ZON) [**Figure 1b**] is mainly produced by *F. graminearum* and *F. culmorum*, consequently co-occurrence with DON and wide geographical spread is described. The production, mainly in maize, wheat, oats, barley, depends on environmental conditions and is favoured by high humidity and low temperature [**1**], [**2**].

DON, ZON and T-2 are ordered in category 3 (not classified relating to carcinogenicity for humans) by the IARC [**3**].

Commission Regulation (EC) No. 1881/2006 [**4**] lays down maximum limits for DON and ZON in cereal grains and cereal-based products intended for human consumption. A combined limit for T-2 and HT-2 will be introduced in the near future. The European Commission also sets guideline limits for DON and ZON in animal feed in Commission Recommendation (2006/576/EC) [**5**], [**6**].

3. Scope

As stated in Article 32 of Regulation (EC) No 882/2004 [**7**], one of the core duties of the EU-RL is to organise interlaboratory comparison tests (ILCs) for the benefit of staff from NRLs. The scope of this ILC was to test the competence of the appointed NRLs to determine the amount of DON, ZON, T-2, HT-2 in cereal samples.

IRMM organised a proficiency test on DON in 2008 [**8**] and on T-2/HT-2 in 2009 [**9**] in cereal products. This year's PT was the first one to be conducted for the determination of ZON.

All invited laboratories were free to use their method of choice. The methodologies used for the determination of these mycotoxins range from high-performance liquid chromatography (HPLC) with various detection systems, over gas chromatography and enzyme linked immunosorbant assays (ELISA). The most common approach in EU member states is however HPLC with mass selective detection.

The ILC was designed and the reported data were processed along the lines of the International Harmonized Protocol for the Proficiency Testing of Analytical Chemical Laboratories [**10**].

As accredited according to ISO 17043 PT provider, EURL-Mycotoxins performed the assessment of the measurement results on the basis of requirements laid down in legislation and followed administrative and logistic procedures of ISO 17043 [**11**].

3.1. Confidentiality

Confidentiality of the participants and their results towards third parties is guaranteed.

4. Time frame

The ILC was agreed upon by the NRL network at the sixth EU-RL Mycotoxins workshop held on 7 April 2011. Specific details of the exercise were refined during the seventh EU-RL Mycotoxins workshop held on 26-27 April 2012 and the planned ILC was published on the IRMM web page [**12**]. The exercise was open for registration on 3 May 2012 [**Annex 13.1**]. The samples were dispatched to the participants on 30 May 2012 [**Annex 13.2**].. Reporting deadline was 5 July 2012.

5. Material

5.1. Preparation

The test materials used in this study were prepared by Eurofins WEJ, Hamburg, Germany. The materials were provided milled to a particle size < 500 µm.
The composition of the test materials and the percent content are the following:
- Sample A: soya (16%), sugar beet (8%), maize gluten (18%), bean (8%), rice (24%), oat (26%)
- Sample B: rye (25%), wheat (17%), maize (17%), oat (8%), rice (33%)

5.2. Homogeneity

To verify the homogeneity of the test materials 10 units per material Sample A and Sample B were selected at random. Two independent determinations per unit were performed with an LC-MS/MS based method, which has been validated at a collaborative trial organised by the EU-RL Mycotoxin group. The measurement batch order was randomised. Sufficient homogeneity was assumed if the between-sample variance (s^2_{sam}) was smaller than a critical factor (c) [**10**].

The between-sample variance (s^2_{sam}) and the within-sample variance (s^2_{an}) were obtained from one-way analysis of variance (ANOVA). The allowable variance (σ^2_{all}) was calculated as $(0.3 * \sigma_p)^2$ from the Horwitz equation modified by Thompson [**13**].

Annex 13.3 lists the details of the homogeneity tests for the two materials. For all materials the between-sample variance (s^2_{sam}) was smaller than the critical factor (c) and, therefore, sufficient homogeneity was assumed.

5.3. Stability

The amount of DON, ZON, T-2 and HT-2 in the test materials were monitored (n=20) over a period of two years (from August 2009 until August 2011) because the material was used as QC-sample. No indication of any degradation was found and the material is considered to be stable.

5.4. Distribution

All samples were packed in cardboard boxes and sent to the participant via DHL express mail. One set of material was sent to every participant. The test materials were dispatched to the participants by IRMM on 30 May 2012. The samples were mostly received within 24 hours after dispatch.

Each participant received:
a) two packages containing approximately 100 g of test materials,
b) an accompanying letter with instructions on sample handling and reporting [**Annex 13.2**],
c) a sample receipt form [**Annex 13.4**] and
d) a registration key for the reporting interface.

The materials were shipped at room temperature; storage upon arrival was required to be at -18° C until the analysis was performed. Based on previous experience a short period of 1-2 days without cooling imposes no harm for the material, for storage above -18° C over a longer period of time no stability information is available.

6. Instructions to participants

The laboratories were asked to report the recovery corrected value and the measurement uncertainty in µg/kg, the coverage factor and the recovery in %.

The results were to be reported in a special online form for which each participant received an individual access code. A specific questionnaire was attached to this online form. The questionnaire was intended to

provide further information on the measurements and the laboratories. A copy of the questionnaire is presented in **Annex 13.5**.

7. Reference values and their uncertainties

Assigned values and their uncertainties for the test samples were established by "Exact-matching Double Isotope Dilution Mass Spectrometry" at IRMM. This methodology is considered to be a primary ratio method with a direct link to SI units [**14**]. The details of the procedure can be found in the report of the NRL PT from 2011.

8. Evaluation of results

8.1. General observations

Thirty-five laboratories, NRL's from twenty-seven MS (two different NRLs for food and feed for eight MS) registered to the PT [**Figure 2**] and all of them sent back results.
34 (Sample A) & 34 (Sample B) sets of results were reported for deoxynivalenol, 33 & 32 for zearalenone, 32 & 28 for T-2 and 30 & 28 for HT-2.

8.2. Scores and evaluation criteria

Individual laboratory performance is expressed in terms of z and zeta (ζ) scores in accordance with ISO 13528 [**15**] and the International Harmonised Protocol [**10**].

$$z = \frac{x_{lab} - X_{ref}}{\sigma_p} \qquad \textbf{Equation 1.}$$

$$\zeta = \frac{x_{lab} - X_{ref}}{\sqrt{u^2_{lab} + u^2_{ref}}} \qquad \textbf{Equation 2.}$$

where:
x_{lab} is the measurement result reported by a participant
X_{ref} is the reference value (assigned value)
u_{lab} is the standard uncertainty reported by a participant
u_{ref} is the standard uncertainty of the reference value
σ_p is the standard deviation for proficiency assessment (target standard deviation)

σ_p was calculated using the Horwitz equation:

- for analyte concentrations < 120 ppb *(ZON Sample B, T-2 Sample A, T-2 Sample B, HT-2 Sample B)*

$$\sigma_p = 0.22 \cdot c \qquad \textbf{Equation 3.}$$

- for analyte concentrations ≥ 120 ppb ≤ 13.8% *(DON Sample A, DON Sample B, ZON Sample A, HT-2 Sample A)*

$$\sigma_p = 0.02 \cdot c^{0.8495} \qquad \textbf{Equation 4.}$$

where:
c = concentration of the measurand (assigned value, $X_{ref,}$) expressed as a dimensionless mass ratio, e.g. 1 ppb = 10^{-9}, 1 ppm = 10^{-6}
The z score compares the participant's deviation from the reference value with the target standard deviation accepted for the proficiency test, σ_p. The z-score is interpreted as:

$\|z\| \leq 2$	satisfactory result
$2 < \|z\| \leq 3$	questionable result
$\|z\| > 3$	unsatisfactory result

The zeta (ζ) score provides an indication of whether the participant's estimate of uncertainty is consistent with the observed deviation from the assigned value. The ζ-score is the most relevant evaluation parameter, as it includes all parts of a measurement result, namely the expected value, its uncertainty as well as the uncertainty of the assigned values.

The interpretation of the zeta score is similar to the interpretation of the z-score:

$\|\zeta\| \leq 2$	satisfactory result
$2 < \|\zeta\| \leq 3$	questionable result
$\|\zeta\| > 3$	unsatisfactory result

An unsatisfactory $|\zeta|$-score might be due to an underestimation of the uncertainty, or to a large error causing a large deviation from the reference value, or to a combination of the two factors. A laboratory with an unsatisfactory $|\zeta|$-score indicated an uncertainty which is not consistent with the laboratory's deviation from the reference value.

8.3. Laboratory results and scoring

Statistical evaluation of the results was performed using MS Excel.

The robust mean values and robust standard deviations were computed according to Algorithm A of ISO 13528 [**15**] by application of a MS Excel macro that was written by the Analytical Methods Committee of The Royal Society of Chemistry (AMC) [**16**].

As a result z-scoring and zeta-scoring was only made for DON and ZON and is in line with the planning to only benchmark results submitted for DON and ZON, unsatisfactory z-scores will results in a corrective action for these two mycotoxins.

The results from the T-2 and HT-2 measurements are nonetheless summarized (for information only) without any z-scoring or further evaluation. This will be done once sufficient experimental data or other evidence can lead to a sound scientific explanation of the discrepancy between IDMS certification and consensus value. The findings will be published as an addendum to this report and shall be discussed with the NRLs at the next possible occasion.

The results as reported by the participants were summarised in **Table 2,4,6,8** together with the z-scores and zeta-scores. Summary of the statistical evaluation for each analyte and test sample are presented in **Tables 1,3,5,7.**

Figures 2-9 provide for each analyte/matrix combinations the individual laboratories values and their uncertainty as reported.

Table 1: Summary statistics for the deoxynivalenol (DON)

		Sample A	Sample B
Number of results		34	34
Range of results	µg/kg	391.6-897	86.5-448.9
Median of results of participants	µg/kg	583.9	266
Mean of results of participants	µg/kg	587.0	267.9
Robust mean of results of participants	µg/kg	573.3	267.2
Assigned value	µg/kg	**605**	**282**
Expanded uncertainty (k=2) of the assigned value	µg/kg	49	26
Robust standard deviation ($\hat{\sigma}$)	µg/kg	109	37
Target standard deviation (fitness for purpose)	µg/kg	104.4	54.6
Number (percentage) of results of |z| > 2.0		1 (3%)	4 (12%)
Number (percentage) of results of |ζ| > 2.0		11 (32%)	6 (18%)

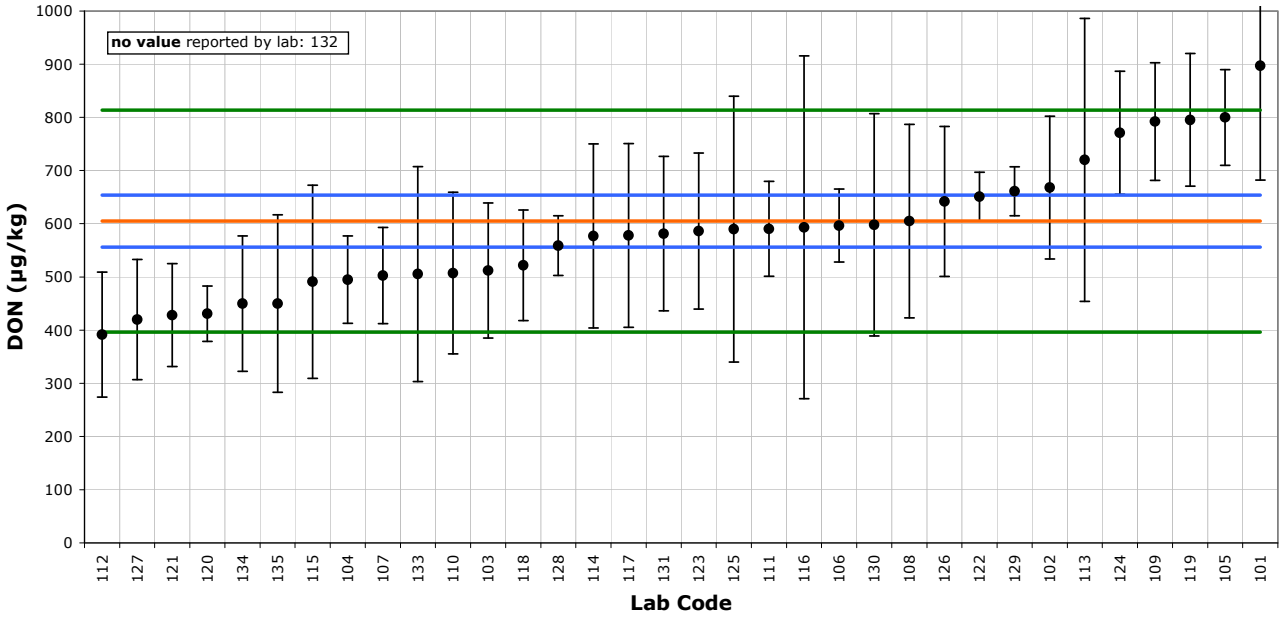
Table 2: Results of analysis, z-scores and zeta-scores for deoxynivalenol (DON)
(The meaning of colors: green – satisfactory, yellow – questionable, red – unsatisfactory result)

Lab Code	SAMPLE A			SAMPLE B		
	Result [µg/kg]	z-score	zeta-score	Result [µg/kg]	z-score	zeta-score
101	**897**	2.8	2.6	**279**	-0.1	-0.1
102	**668**	0.6	0.9	**295**	0.2	0.4
103	**512**	-0.9	-1.4	**246**	-0.7	-1.0
104	**495**	-1.1	-2.3	**106.8**	-3.2	-11.1
105	**800**	1.9	3.8	**400**	2.2	4.7
106	**596.5**	-0.1	-0.2	**448.9**	3.1	5.8
107	**502.7**	-1.0	-2.0	**86.5**	-3.6	-12.7
108	**605**	0.0	0.0	**255**	-0.5	-0.7
109	**792.4**	1.8	3.1	**321.94**	0.7	1.5
110	**507**	-0.9	-1.2	**266**	-0.3	-0.4
111	**590.5**	-0.1	-0.3	**275.1**	-0.1	-0.2
112	**391.6**	-2.0	-3.4	**213.6**	-1.3	-2.0
113	**720**	1.1	0.9	**340**	1.1	0.9
114	**577**	-0.3	-0.3	**260**	-0.4	-0.5
115	**491**	-1.1	-1.2	**240**	-0.8	-0.9
116	**593.4**	-0.1	-0.1	**264.4**	-0.3	-0.3
117	**578**	-0.3	-0.3	**268**	-0.3	-0.3
118	**522**	-0.8	-1.4	**245**	-0.7	-1.3
119	**795.3**	1.8	2.8	**325.9**	0.8	1.4
120	**431**	-1.7	-4.9	**241**	-0.8	-2.1
121	**428.3**	-1.7	-3.3	**268.4**	-0.2	-0.4
122	**651.1**	0.4	1.4	**286.4**	0.1	0.3
123	**586.2**	-0.2	-0.2	**252.2**	-0.5	-0.9
124	**771.2**	1.6	2.6	**327.5**	0.8	1.6
125	**590**	-0.1	-0.1	**286**	0.1	0.1
126	**642**	0.4	0.5	**280**	0.0	-0.1
127	**420**	-1.8	-3.0	**348**	1.2	1.4
128	**558.77**	-0.4	-1.2	**256.67**	-0.5	-0.8
129	**661**	0.5	1.7	**297**	0.3	0.9
130	**598**	-0.1	-0.1	**229**	-1.0	-1.3
131	**581.5**	-0.2	-0.3	**248.5**	-0.6	-1.0
132	*No result*			*No result*		
133	**505.5**	-1.0	-1.0	**205.5**	-1.4	-1.8
134	**450**	-1.5	-2.3	**179**	-1.9	-3.6
135	**450**	-1.5	-1.8	**266**	-0.3	-0.3

The results are written as reported by the laboratories.

Figure 2: EU-RL Mycotoxins PT 2012: Deoxynivalenol in cereals - Sample A

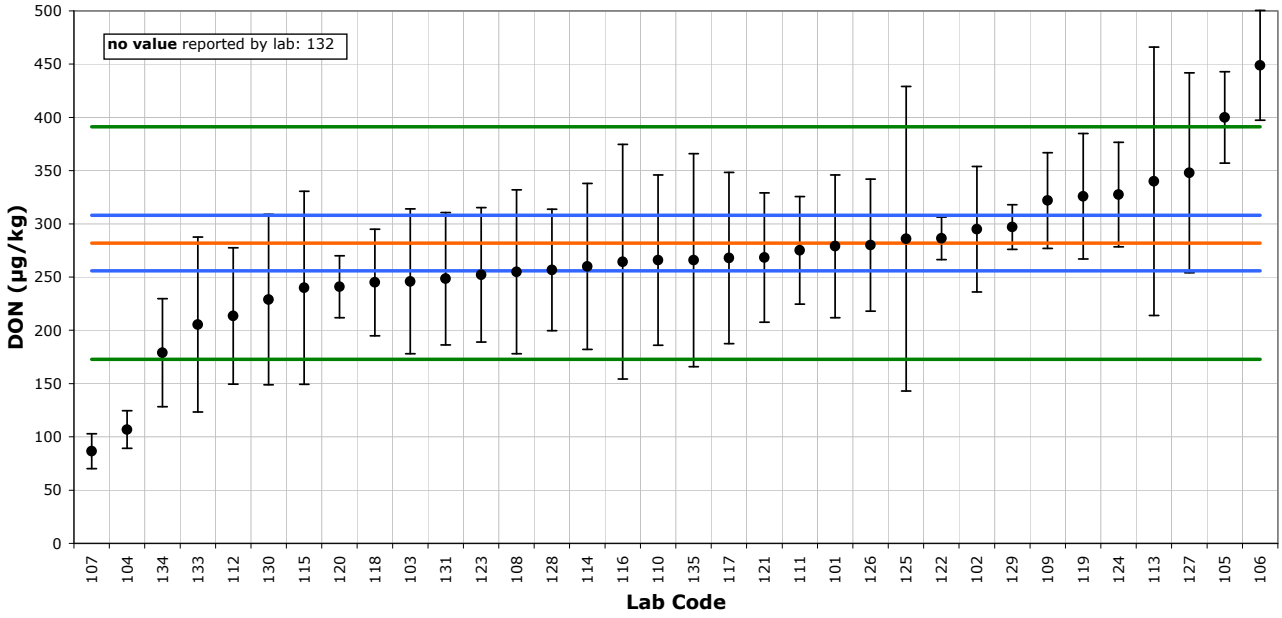
Certified value: Xref = 605 µg/kg; Uref = 49 µg/kg (k=2); σ = 104.4 µg/kg



This graph displays all revised measurement results and their associated uncertainties. The uncertainties are shown as reported.
The red line corresponds to **Xref**, the blue lines mark the boundary of the reference interval **(Xref ± 2uref)**, and the green lines that of the target interval **(Xref ± 2σ)**.

Figure 3: EU-RL Mycotoxins PT 2012: Deoxynivalenol in cereals - Sample B

Certified value: Xref = 282 µg/kg; Uref = 26 µg/kg (k=2); σ = 54.6 µg/kg



This graph displays all revised measurement results and their associated uncertainties. The uncertainties are shown as reported.
The red line corresponds to **Xref**, the blue lines mark the boundary of the reference interval **(Xref ± 2uref)**, and the green lines that of the target interval **(Xref ± 2σ)**.

Table 3: Summary statistics for the zearalenone (ZON)

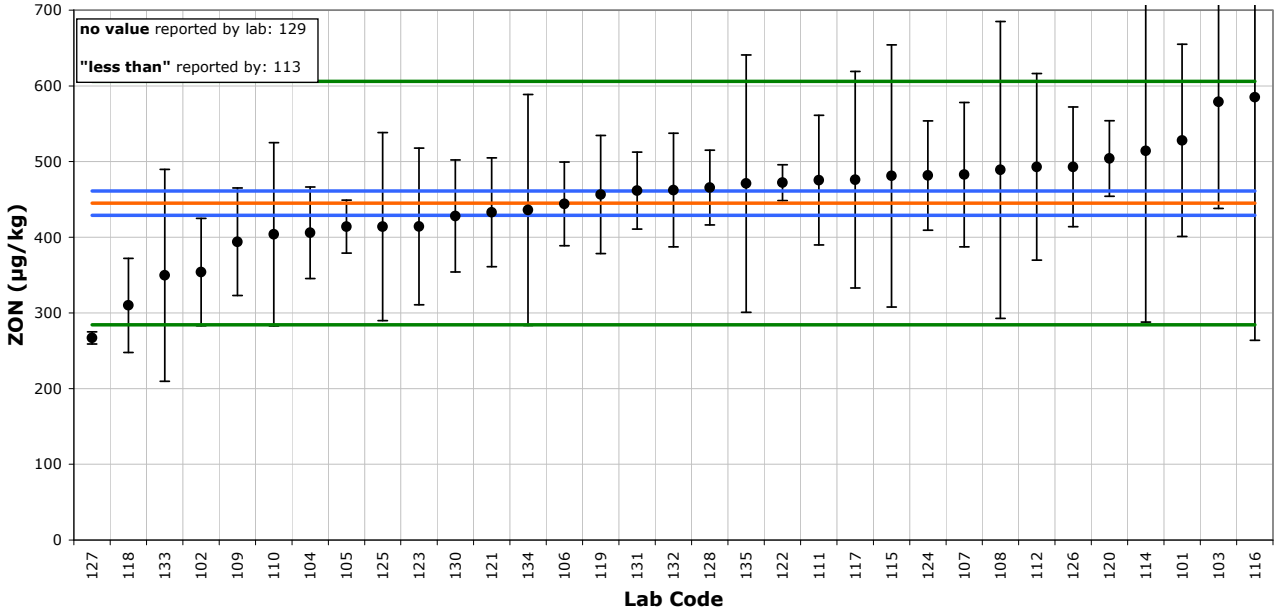
		Sample A	Sample B
Number of results		33	32
Range of results	µg/kg	267-585	20.5-39.7
Median of results of participants	µg/kg	462.2	30.1
Mean of results of participants	µg/kg	449.7	29.7
Robust mean of results of participants	µg/kg	457.8	29.8
Assigned value	µg/kg	**445**	**28**
Expanded uncertainty (k=2) of the assigned value	µg/kg	16	4
Robust standard deviation ($\hat{\sigma}$)	µg/kg	47.6	3.8
Target standard deviation (fitness for purpose)	µg/kg	80.4	6.2
Number (percentage) of results of |z| > 2.0		1 (3%)	0 (0%)
Number (percentage) of results of |ζ| > 2.0		4 (12%)	3 (9%)

Table 4: Results of analysis, z-scores and zeta-scores for zearalenone (ZON)
(The meaning of colours: green – satisfactory, yellow – questionable, red – unsatisfactory result)

Lab Code	SAMPLE A			SAMPLE B		
	Result [µg/kg]	z-score	zeta-score	Result [µg/kg]	z-score	zeta-score
101	**528**	1.0	1.3	**23**	-0.8	-1.4
102	**354**	-1.1	-2.5	**25.7**	-0.4	-0.7
103	**579**	1.7	1.9	**32**	0.6	0.6
104	**406**	-0.5	-1.2	<25		
105	**414**	-0.4	-1.6	**33**	0.8	2.0
106	**444.1**	0.0	0.0	**37.5**	1.5	3.1
107	**482.7**	0.5	0.8	**33.2**	0.8	1.3
108	**489**	0.5	0.4	**23**	-0.8	-1.0
109	**393.93**	-0.6	-1.4	**22.13**	-1.0	-1.5
110	**404**	-0.5	-0.7	**29**	0.2	0.2
111	**475.5**	0.4	0.7	**20.5**	-1.2	-2.8
112	**493**	0.6	0.8	**30.2**	0.4	0.5
113	<3			**39.7**	1.9	2.0
114	**514**	0.9	0.6	**30**	0.3	0.3
115	**481**	0.4	0.4	**30.5**	0.4	0.5
116	**585**	1.7	0.9	**39.6**	1.9	1.9
117	**476**	0.4	0.4	**30.3**	0.4	0.5
118	**310**	-1.7	-4.2	<25		
119	**456.4**	0.1	0.3	**29.2**	0.2	0.3
120	**504**	0.7	2.2	**32**	0.6	1.6
121	**433**	-0.1	-0.3	**26.4**	-0.3	-0.5
122	**472.1**	0.3	1.9	**32.1**	0.7	1.9
123	**414.3**	-0.4	-0.6	**30.9**	0.5	0.4
124	**481.6**	0.5	1.0	**24.5**	-0.6	-1.3
125	**414**	-0.4	-0.5	**29**	0.2	0.2
126	**493**	0.6	1.2	**31.5**	0.6	1.1
127	**267**	-2.2	-19.9	**26.5**	-0.2	-0.7
128	**465.56**	0.3	0.8	**32.82**	0.8	2.3
129	*No result*			*No result*		
130	**428**	-0.2	-0.4	**28**	0.0	0.0
131	**461.5**	0.2	0.6	**31.1**	0.5	1.2
132	**462.2**	0.2	0.4	**29.5**	0.2	0.4
133	**349.7**	-1.2	-1.4	**25**	-0.5	-0.6
134	**436**	-0.1	-0.1	**33**	0.8	0.8
135	**471**	0.3	0.3	**29.9**	0.3	0.4

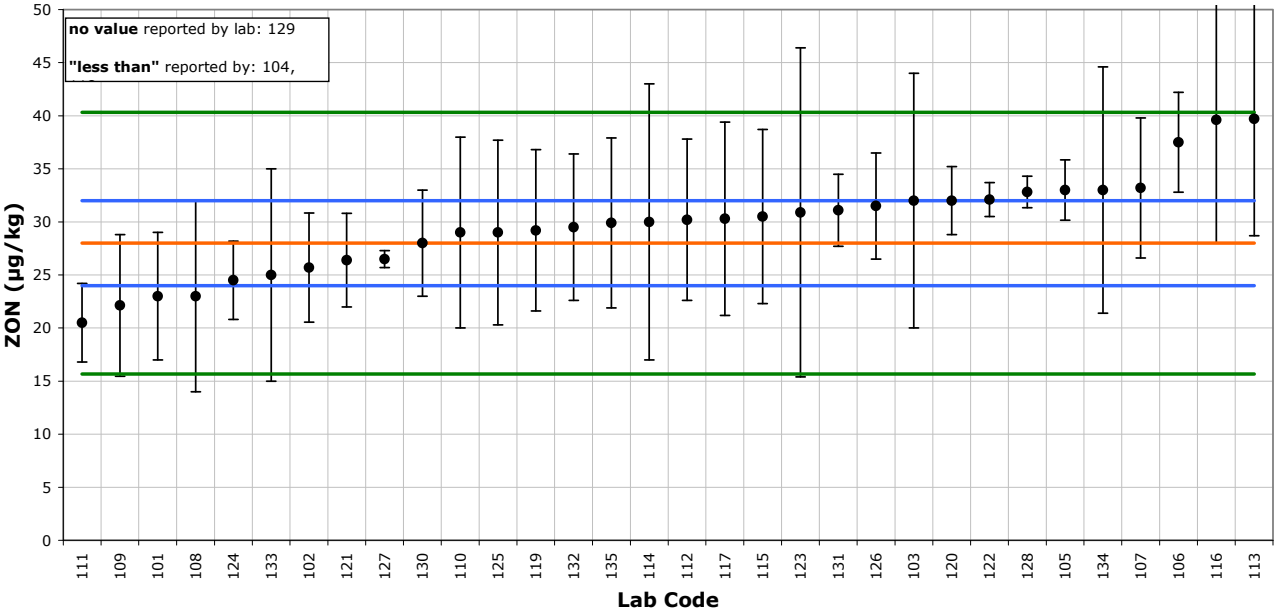
The results are written as reported by the laboratories.

Figure 4: EU-RL Mycotoxins PT 2012: Zearalenone in cereals - Sample A
Certified value: Xref = 445 µg/kg; Uref = 16 µg/kg (k=2); σ = 80.4 µg/kg



This graph displays all revised measurement results and their associated uncertainties. The uncertainties are shown as reported.
The red line corresponds to **Xref**, the blue lines mark the boundary of the reference interval **(Xref ± 2uref)**, and the green lines that of the target interval **(Xref ± 2σ)**.

Figure 5: EU-RL Mycotoxins PT 2012: Zearalenone in cereals - Sample B
Certified value: Xref = 28 µg/kg; Uref = 4 µg/kg (k=2); σ = 6.2 µg/kg



This graph displays all revised measurement results and their associated uncertainties. The uncertainties are shown as reported.
The red line corresponds to **Xref**, the blue lines mark the boundary of the reference interval **(Xref ± 2uref)**, and the green lines that of the target interval **(Xref ± 2σ)**.

Table 5: Summary statistics for the T-2

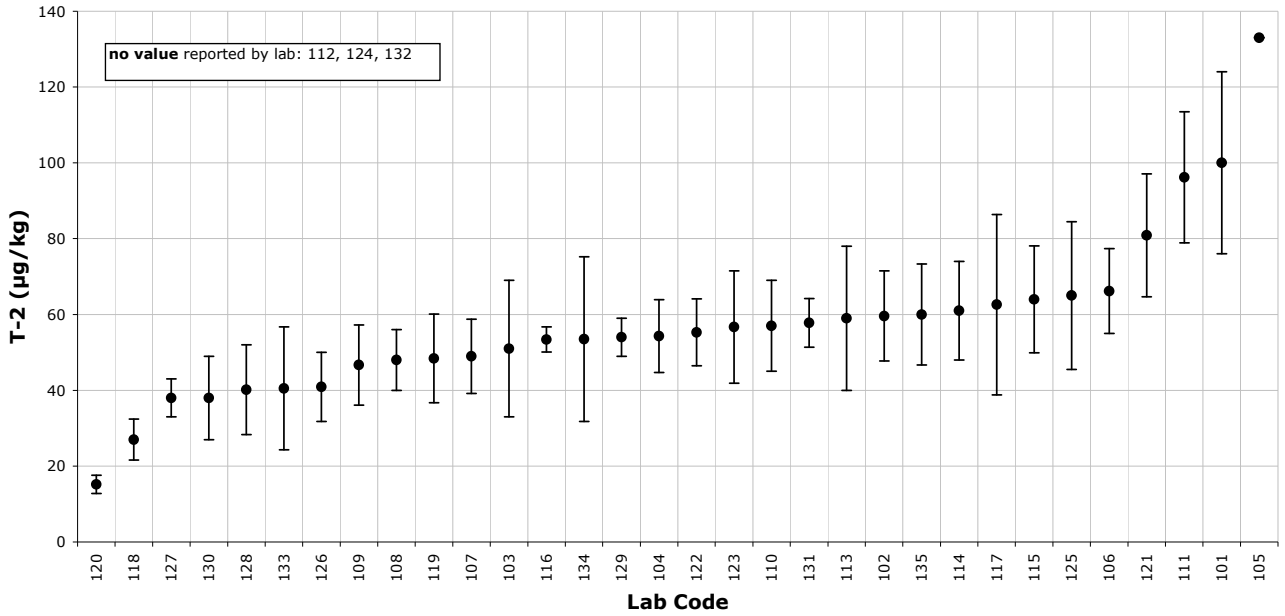
		Sample A	Sample B
Number of results		32	28
Range of results	µg/kg	15.2-133	11.8-60
Median of results of participants	µg/kg	54.8	27.3
Mean of results of participants	µg/kg	57.3	27.7
Robust mean of results of participants	µg/kg	54.7	26.4
Robust standard deviation ($\hat{\sigma}$)	µg/kg	11.5	5.2
Target standard deviation	µg/kg	11.4	4.0

Table 6: Results of analysis (T-2)

Lab Code	SAMPLE A	SAMPLE B
101	100	<20
102	59.6	27.5
103	51	27
104	54.3	20.7
105	133	60
106	66.17	28.7
107	49	30.6
108	48	22
109	46.66	34.44
110	57	25
111	96.2	46
112	*No result*	*No result*
113	59	30
114	61	28
115	64	<50
116	53.4	23.6
117	62.6	31.4
118	27	11.8
119	48.4	30.1
120	15.2	33
121	80.9	<20
122	55.28	26.08
123	56.7	27.6
124	*No result*	*No result*
125	65	28
126	40.9	25.6
127	38	19.4
128	40.14	30.55
129	54	24
130	38	19
131	57.8	25.8
132	*No result*	*No result*
133	40.5	18.5
134	53.5	22.5
135	60	<50

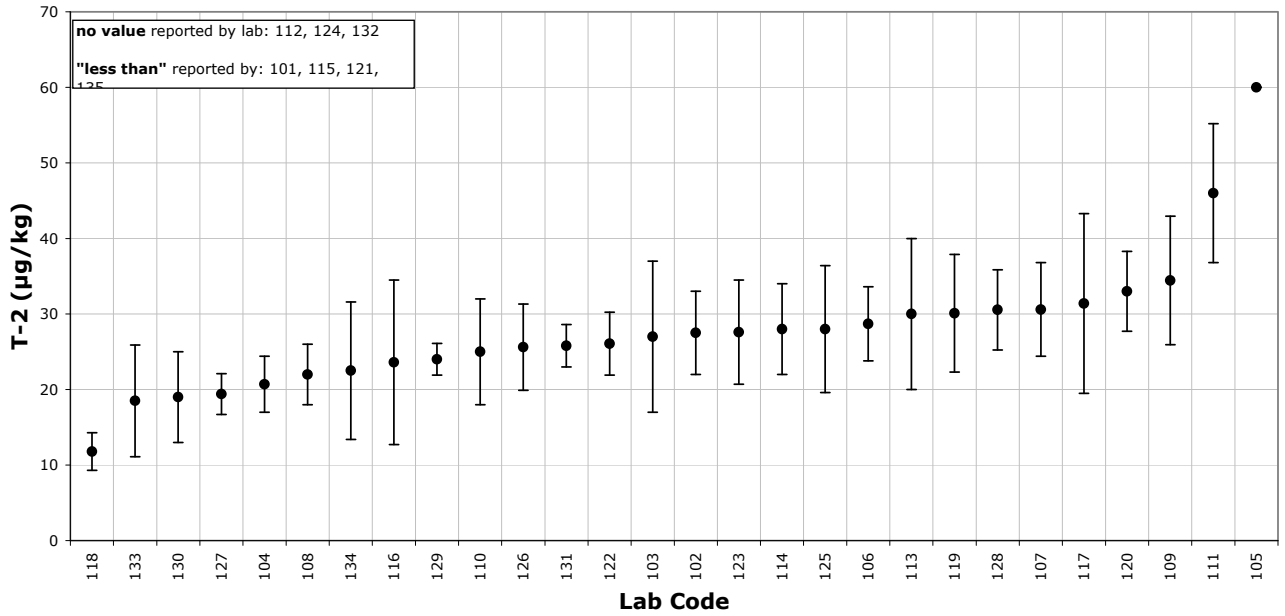
The results are written as reported by the laboratories.

Figure 6: EU-RL Mycotoxins PT 2012: T-2 in cereals - Sample A



This graph displays all revised measurement results and their associated uncertainties. The uncertainties are shown as reported.

Figure 7: EU-RL Mycotoxins PT 2012: T-2 in cereals - Sample B



This graph displays all revised measurement results and their associated uncertainties. The uncertainties are shown as reported.

Table 7: Summary statistics for the HT-2

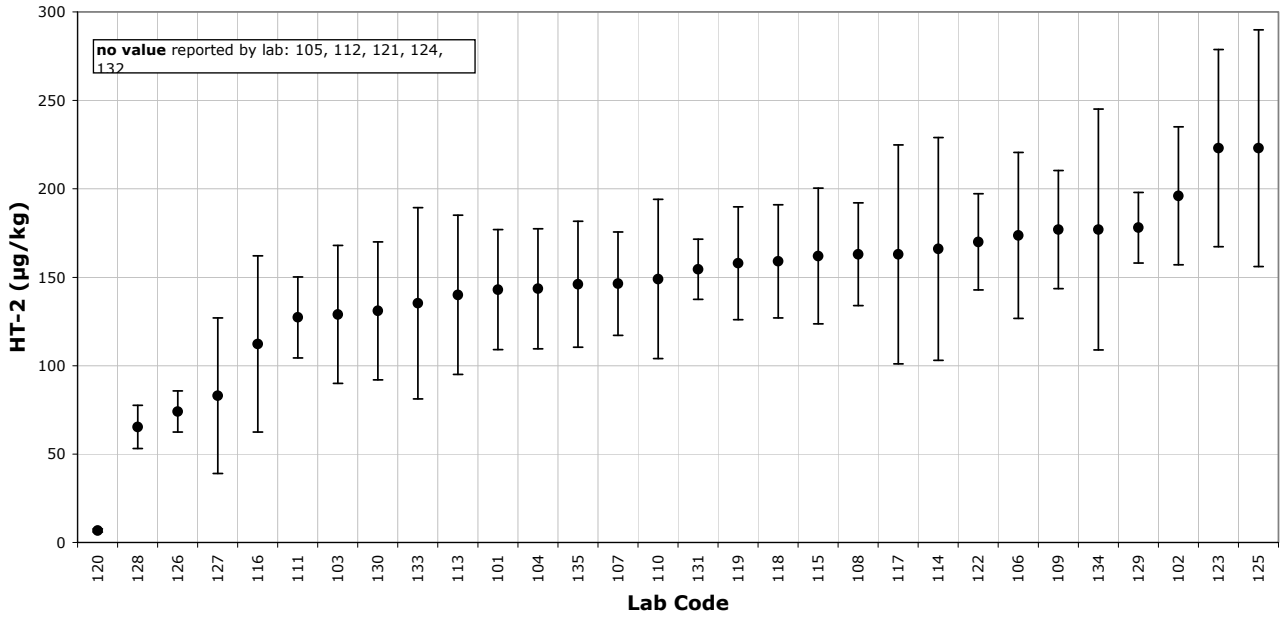
		Sample A	Sample B
Number of results		30	28
Range of results	µg/kg	6.8-223	12.3-70.5
Median of results of participants	µg/kg	151.8	41
Mean of results of participants	µg/kg	145.8	40.3
Robust mean of results of participants	µg/kg	156.6	40
Robust standard deviation ($\hat{\sigma}$)	µg/kg	28.1	10.1
Target standard deviation	µg/kg	40.8	10.9

Table 8: Results of analysis (HT-2)

Lab Code	SAMPLE A	SAMPLE B
101	143	27
102	196	46.7
103	129	47
104	143.5	42.5
105	*No result*	*No result*
106	173.68	32.9
107	146.4	40.7
108	163	45
109	177	70.5
110	149	34
111	127.3	19.7
112	*No result*	*No result*
113	140	41
114	166	41
115	162	<50
116	112.3	27.7
117	163	43.2
118	159	34.9
119	157.9	51.8
120	6.8	52
121	*No result*	<20
122	170.0	58.4
123	223	34.2
124	*No result*	*No result*
125	223	58
126	74.1	30.2
127	83	12.3
128	65.39	41.09
129	178	48
130	131	41
131	154.5	38.5
132	*No result*	*No result*
133	135.3	31.5
134	177	36.5
135	146	<50

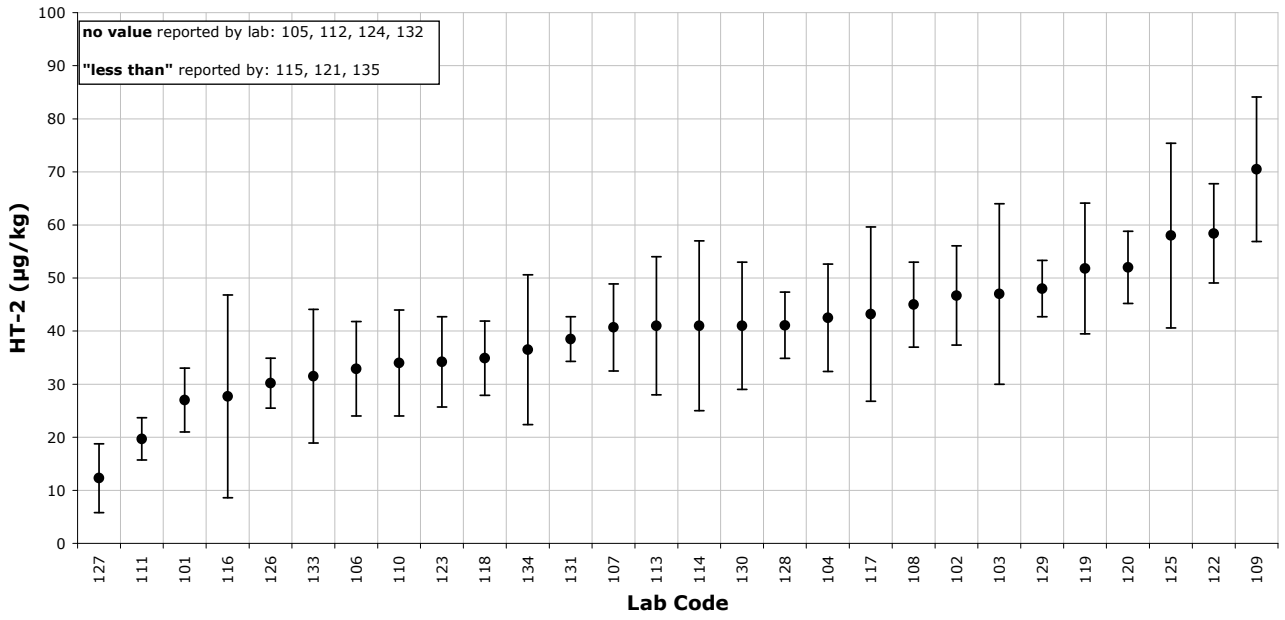
The results are written as reported by the laboratories.

Figure 8: EU-RL Mycotoxins PT 2012: HT-2 in cereals - Sample A



This graph displays all revised measurement results and their associated uncertainties. The uncertainties are shown as reported.

Figure 9: EU-RL Mycotoxins PT 2012: HT-2 in cereals - Sample B



This graph displays all revised measurement results and their associated uncertainties. The uncertainties are shown as reported.

8.4. Evaluation of the questionnaire

All laboratories that reported results, in total thirty four participants, supplied their filled in questionnaire. Summary of the answers is presented in the **Annex 13.6.**

General overview of the reported answers showed that participants used mainly three techniques – HPLC-DAD, HPLC-FLD and LC-MS/MS - for obtaining the results for different mycotoxins.

For the determination of T-2 and HT-2, most of the laboratories (80%) used LC-MS/MS. HPLC-FLD was applied for ZON by 73% of the participants. Regarding the analysis of DON, LC-MS/MS and HPLC-DAD techniques were used equally.

Fifty percent of the participants used Biopure standard for the determination of DON, 47% for ZON, 62% for T-2 and 61% for HT-2.

Most of the laboratories analysed 50-150 samples or more for DON and ZON, but less than 50 samples for T-2 and HT-2 annually. Eighty-nine percent of the NRLs are accredited for the analysis of DON, 80% for ZON and only 51% for both T-2 and HT-2.

For the recovery estimation nearly all of the participants used a "Standard solution to blank" method.

Details about the applied methodology for different analytes – extraction, clean up, overnight stop, etc. - are presented in **Annex 13.6**. No statistically relevant information could be obtained that linked performance results with answers on methodology, overnight stop etc.

All participants found the instructions adequate and regarding the registration-reporting interface the EU-RL received mostly good reviews.

9. Conclusions

34 (Sample A) & 34 (Sample B) sets of results were reported for DON, 33 & 32 for ZON, 32 & 28 for T-2 and 30 & 28 for HT-2.

Most of the participants performed satisfactory or better than the minimal performance criteria required. The performance of most NRLs was very good and better compared with a previous PT for DON [**8**] organised by the EU-RL. This was the first PT conducted for the determination of ZON and the results of most participants were outstanding.

Zeta-scores were not as good as the z-scores, which indicate that the respective participants should review their uncertainty estimation.

It was noted that the consensus values and the certified values match for DON and ZON, but not for T-2 and HT-2 toxins. IRMM has dedicated itself to investigate the reason for this difference as it has shown in previous PTs that IDMS certification is a method with many assets for the generation of assigned values in PTs.

10. Acknowledgements

The organizers of the study would like to thank Franz Ulberth and Beatriz de la Calle for their support.

The laboratories participating in this exercise, listed in [**Table 9**], are also kindly acknowledged.

Table 9: Participating laboratories

Organisation	Country
AGES GmbH	Austria
CODA-CERVA, Chemical Safety Food Chain	Belgium
Central Laboratory for Chemical Testing and Control, Control of Mycotoxins	Bulgaria
Department Of Agriculture, Analytical Laboratories Section	Cyprus
State General Laboratory, Food Contamination Laboratory	Cyprus
Czech Agriculture and Food Inspection Authority	Czech Republic
Central Institute for Testing and Supervising in Agriculture (UKZUZ)	Czech Republic
Ministry of Food, Agriculture and Fisheries; Danish Veterinary and Food Adm.	Denmark
DTU Food, Food Chemistry	Denmark
Agricultural Research Centre, Lab For Residues and Contaminants	Estonia
Finnish Food Safety Authority (Evira), Chemistry and Toxicology Unit	Finland
Finnish Customs Laboratory	Finland
Laboratoire SCL de Rennes, Mycotoxines	France
Federal Institute For Risk Assessment -BfR	Germany
General Chemical State Laboratory, Division of Environment, SectA	Greece
National Food Chain Safety Office, Food and Feed Safety Directorate – Feed Investigation	Hungary
National Food Chain Safety Office, Food and Feed Safety Directorate – Food Investigation	Hungary
Public Analyst's Laboratory, LC-MS	Ireland
Istituto Superiore di Sanità	Italy
Institute of Food Safety, Animal Health and Environment "BIOR"	Latvia
National Food and Veterinary Risk Assessment Institute, Chemistry Department	Lithuania
Laboratoire National de Santé	Luxembourg
Public Health Laboratory	Malta
RIKILT, Institute of Food Safety, Natural Toxins and Pesticides	Netherlands
Veterinary Research Institute, Pharmacology and Toxicology	Poland
National Institute of Public Health - National Institute of Hygiene, Food Safety	Poland
ASAE LSA LFQ	Portugal
Sanitary Veterinary And Food Safety Directorate	Romania
Hygiene Institute of Veterinary Public Health, Mycotoxins	Romania
State Veterinary and Food Institute	Slovakia
University in Ljubljana, Veterinary Faculty-National Veterinary Institute	Slovenia
Centro Nacional De Alimentacion, Unit of Toxins and PAHs	Spain
National Food Agency, Chemical Division 2	Sweden
National Veterinary Institute (SVA), KMF/SFL	Sweden
Food and Environment Research Agency, FES	United Kingdom

11. Abbreviations

ANOVA	Analysis of variance
DON	Deoxynivalenol
EC	European Commission
ELISA	Enzyme linked immunosorbant assays
EU	European Union
EU-RL	European Reference Laboratory
FLD	Fluorescent detection
HPLC	High-performance liquid chromatography
IAC	Immunoaffinity column
IDMS	Isotope Dilution Mass Spectrometry
ILC	Interlaboratory Comparison
IRMM	Institute for Reference Materials and Measurements
ISO	International Organisation for Standardisation
IUPAC	International Union for Pure and Applied Chemistry
JRC	Joint Research Centre
LOD	Limit of Detection
LOQ	Limit of Quantification
NRL	National Reference Laboratory
PT	Proficiency Test
ZON	Zearalenone

12. References

[**1**] Bennett, J. W., & Klich, M. (2003). Mycotoxins. Clinical Microbiology Review, 16(3), 497–516.

[**2**] Desjardins, A. E., Hohn, T. M., & McCormick, S. P. (1993). Trichothecene biosynthesis in Fusarium species: Chemistry, genetics, and significance. Clinical Microbiology Reviews, 57(3), 595–604.

[**3**] Castegnaro M., Barek J., Fremy J.M., Lafontaine M., Sansone E.B. and Telling G.M. Laboratory decontamination and destruction of carcinogens in laboratory wastes: some mycotoxins. IARC Scientific Publication No. 113, International Agency for Research on Cancer, Lyon (France), 1991, p. 63.

[**4**] Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs
http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:2006R1881:20100701:EN:PDF

[**5**] Commission Recommendation (2006/576/EC) of 17 August 2006 on the presence of deoxynivalenol, zearalenone, ochratoxin A, T-2 and HT-2 and fumonisins in products intended for animal feeding
http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006:229:0007:0009:EN:PDF

[**6**] Lerda, D., Mycotoxins Factsheet Fourth Edition – September 2011 – Joint Research Centre
http://irmm.jrc.ec.europa.eu/EURLs/eurl_mycotoxins/Documents/Factsheet%20Mycotoxins.pdf

[**7**] Commission Regulation (EC) No 882/2004 of the European Parliament and of the council of 29 April 2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules
http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:2004R0882:20060525:EN:PDF

[**8**] Stroka, J., Doncheva, I., Breidbach, A., Mischke, C., Report on the 2008 Proficiency Test of the Community Reference Laboratory for Mycotoxins, for the Network of National Reference Laboratories, regarding the Determination of Deoxynivalenol in a Cereal Product and a Test Solution, JRC Scientific and Technical Reports, EUR 23787 EN: 2009
http://irmm.jrc.ec.europa.eu/EURLs/eurl_mycotoxins/interlaboratory_comparisons/Documents/eur_23787_en_don_cereal.pdf

[**9**] Stroka, J., Breidbach, A., Bouten, K., Kroeger, K., Ambrosio, M., Lerda, D., Report on the 2009 Proficiency Test of the Community Reference Laboratory for Mycotoxins, for the Network of National Reference Laboratories, Regarding the Determination of T-2 and HT-2 Toxins in a Cereal Products, JRC Scientific and Technical Reports, EUR 24315 EN: 2010
http://irmm.jrc.ec.europa.eu/EURLs/eurl_mycotoxins/interlaboratory_comparisons/Documents/eur_24315_en.pdf

[**10**] Thompson, M., Ellison, S.L.R., and Wood, R., The International Harmonised Protocol for the Proficiency Testing of Analytical Chemistry Laboratories. Pure Appl. Chem., 2006. 78(1): p. 145–196.
http://media.iupac.org/publications/pac/2006/pdf/7801x0145.pdf

[**11**] ISO/IEC 17043:2010 - Conformity assessment -- General requirements for proficiency testing

[**12**] IRMM. Inter-laboratory Comparisons at the Institute for Reference Materials and Measurements.; Available from:
http://irmm.jrc.ec.europa.eu/EURLs/EURL_mycotoxins/interlaboratory_comparisons/Pages/index.aspx

[**13**] Thompson, M., Recent trends in inter-laboratory precision at ppb and sub-ppb concentrations in relation to fitness for purpose criteria in proficiency testing, Analyst, 2000, 125, 385-386

[**14**] Mackay, L.G., et al., High accuracy analysis by isotope dilution mass spectrometry using an iterative exact matching technique. Accreditation and Quality Assurance: Journal for Quality, Comparability and Reliability in Chemical Measurement, 2003. 8(5): p. 191-194.

[**15**] ISO 13528:2005; Statistical Methods for Use in Proficiency Testing by Interlaboratory Comparisons

[**16**] Analytical Methods Committee, Robust statistics: a method of coping with outliers, Technical brief No 6, Apr 2001.
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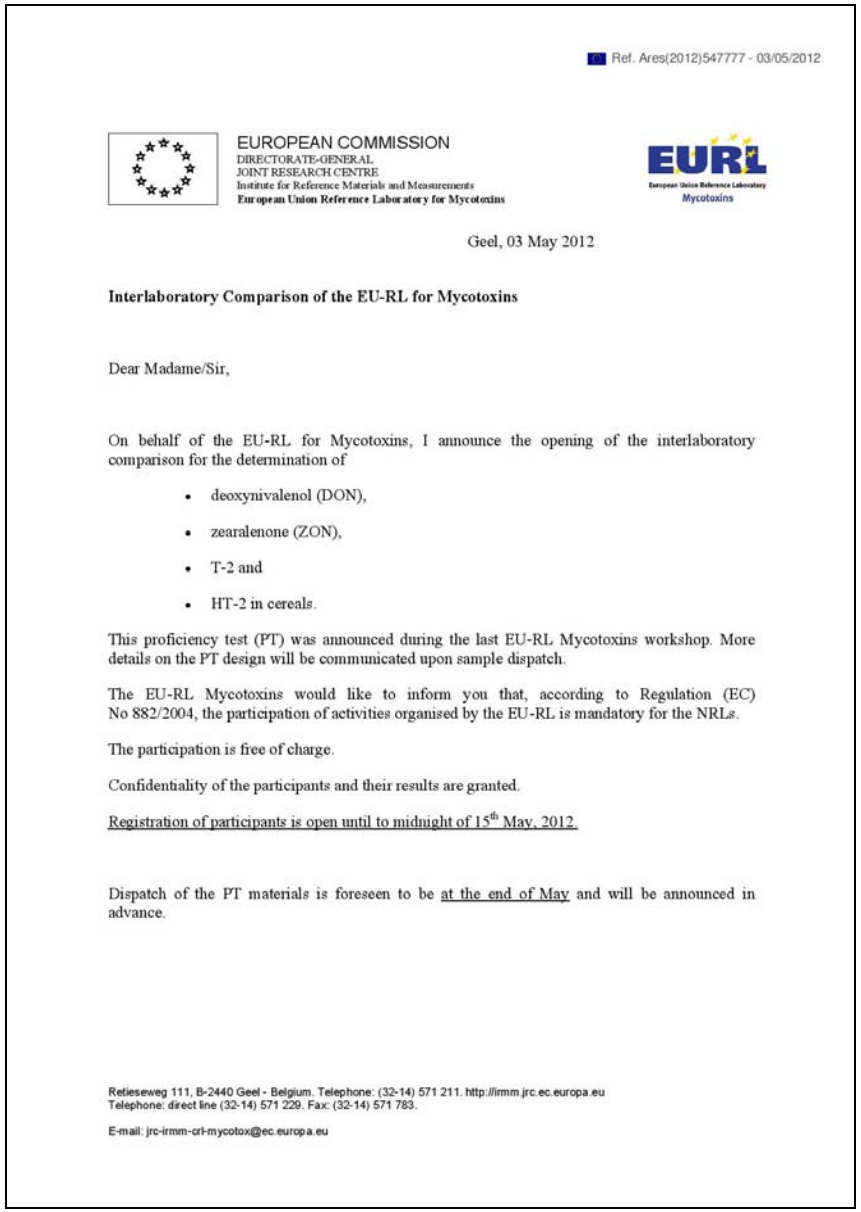
13. Annexes

13.1. Opening of registration



Ref. Ares(2012)547777 - 03/05/2012

EUROPEAN COMMISSION
DIRECTORATE-GENERAL
JOINT RESEARCH CENTRE
Institute for Reference Materials and Measurements
European Union Reference Laboratory for Mycotoxins

EURL
European Union Reference Laboratory
Mycotoxins

Geel, 03 May 2012

Interlaboratory Comparison of the EU-RL for Mycotoxins

Dear Madame/Sir,

On behalf of the EU-RL for Mycotoxins, I announce the opening of the interlaboratory comparison for the determination of

- deoxynivalenol (DON),
- zearalenone (ZON),
- T-2 and
- HT-2 in cereals.

This proficiency test (PT) was announced during the last EU-RL Mycotoxins workshop. More details on the PT design will be communicated upon sample dispatch.

The EU-RL Mycotoxins would like to inform you that, according to Regulation (EC) No 882/2004, the participation of activities organised by the EU-RL is mandatory for the NRLs.

The participation is free of charge.

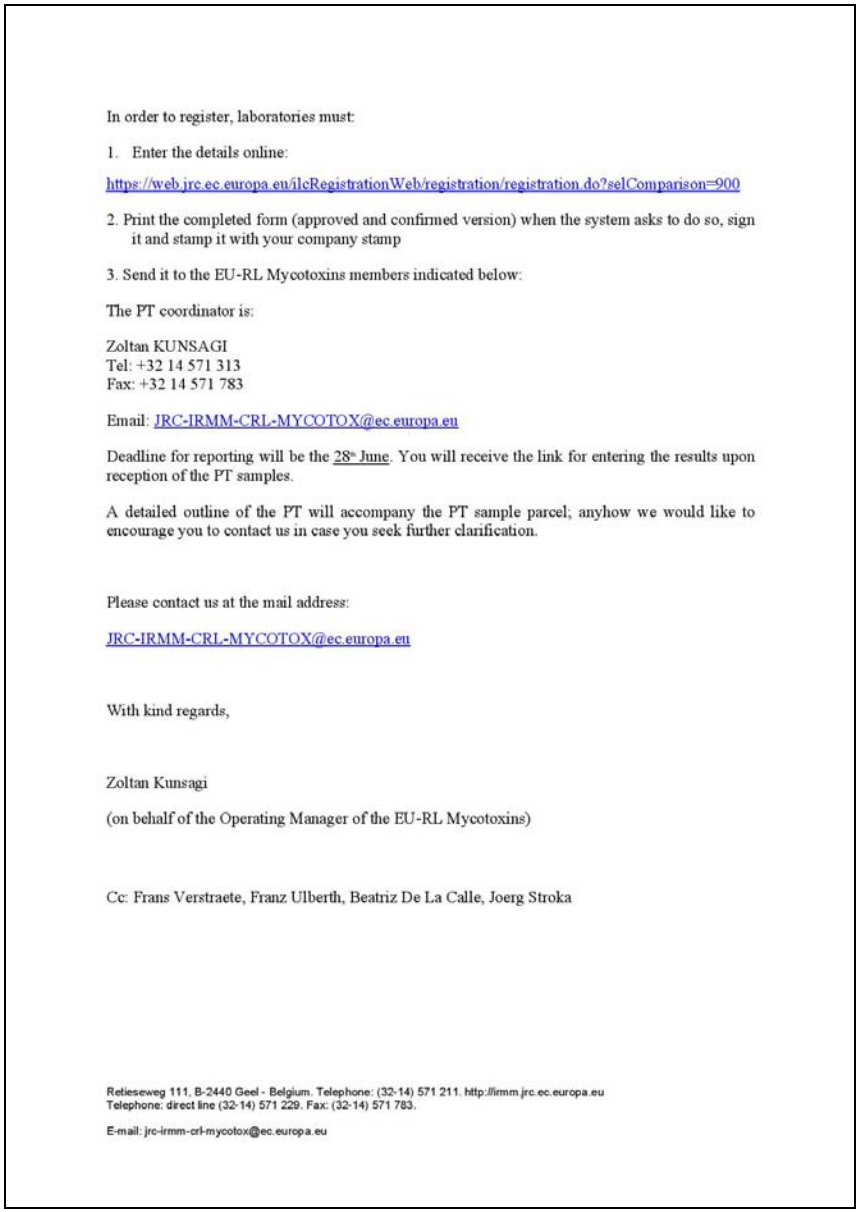
Confidentiality of the participants and their results are granted.

Registration of participants is open until to midnight of 15th May, 2012.

Dispatch of the PT materials is foreseen to be at the end of May and will be announced in advance.

Retieseweg 111, B-2440 Geel - Belgium. Telephone: (32-14) 571 211. http://irmm.jrc.ec.europa.eu
Telephone: direct line (32-14) 571 229. Fax: (32-14) 571 783.

E-mail: jrc-irmm-crl-mycotox@ec.europa.eu



In order to register, laboratories must:

1. Enter the details online:

https://web.jrc.ec.europa.eu/ilcRegistrationWeb/registration/registration.do?selComparison=900

2. Print the completed form (approved and confirmed version) when the system asks to do so, sign it and stamp it with your company stamp

3. Send it to the EU-RL Mycotoxins members indicated below:

The PT coordinator is:

Zoltan KUNSAGI
Tel: +32 14 571 313
Fax: +32 14 571 783

Email: JRC-IRMM-CRL-MYCOTOX@ec.europa.eu

Deadline for reporting will be the 28th June. You will receive the link for entering the results upon reception of the PT samples.

A detailed outline of the PT will accompany the PT sample parcel; anyhow we would like to encourage you to contact us in case you seek further clarification.

Please contact us at the mail address:

JRC-IRMM-CRL-MYCOTOX@ec.europa.eu

With kind regards,

Zoltan Kunsagi

(on behalf of the Operating Manager of the EU-RL Mycotoxins)

Cc: Frans Verstraete, Franz Ulberth, Beatriz De La Calle, Joerg Stroka

Retieseweg 111, B-2440 Geel - Belgium. Telephone: (32-14) 571 211. http://irmm.jrc.ec.europa.eu
Telephone: direct line (32-14) 571 229. Fax: (32-14) 571 783.

E-mail: jrc-irmm-crl-mycotox@ec.europa.eu

13.2. Accompanying letter

Ref. Ares(2012)621557 - 24/05/2012

EUROPEAN COMMISSION
DIRECTORATE-GENERAL
JOINT RESEARCH CENTRE
Institute for Reference Materials and Measurements
European Union Reference Laboratory for Mycotoxins

EURL
European Union Reference Laboratory
Mycotoxins

Geel, 30 May 2012

Ref: 2012 Proficiency Testing of National Reference Laboratories (NRLs) and official control laboratories on DON, ZON, T-2 and HT-2 in cereal samples

Dear Participant,

Please read the following information carefully before starting any analysis. If there are additional questions, do not hesitate to contact us by either phone or email (see details below).

The 2012 PT aims to:

Assess the content in two naturally contaminated test samples (marked as "Sample A", "Sample B"). You will be asked to report the **recovery corrected value** (µg/kg), including your **recovery** (%) and **measurement uncertainty** (µg/kg) for a coverage factor of 2 (k=2).

Please confirm the parcel's receipt by fax or e-mail immediately, by using the "**Materials receipt form**". If any material is damaged, please request new material immediately.

The materials are shipped at room temperature; storage however should be at -18° C until the analysis is performed. A short period of 1-2 days without cooling is no harm for the material, but a longer period of storage above -18° C shall be avoided.

Please report all requested results and answer the questionnaire at
https://web.jrc.ec.europa.eu/ilcReportingWeb
The password key for this interface is included in the parcel with the test materials. When you enter the code please pay attention to the capital letters!

Retieseweg 111, B-2440 Geel - Belgium. Telephone: (32-14) 571 211. http://irmm.jrc.ec.europa.eu
Telephone: direct line (32-14) 571 229. Fax: (32-14) 571 783.

E-mail: jrc-irmm-crl-mycotox@ec.europa.eu

Print out the final pdf and return the signed and stamped report sheet NOT later than **5th July 2012** to:

Zoltan Kunsagi
JRC-IRMM-FSQ
EURL Mycotoxins
Retieseweg 111
B-2440 Geel, Belgium
Tel: +32-14-571 313
FAX: +32-14-571 783
E-mail: Jrc-irmm-crl-mycotox@ec.europa.eu

In case of questions please do not hesitate to contact us.

Zoltan Kunsagi
(on behalf of the Operating Manager of the EU-RL Mycotoxins)

Cc: Frans Verstraete, Franz Ulberth, Beatriz De La Calle, Joerg Stroka

Retieseweg 111, B-2440 Geel - Belgium. Telephone: (32-14) 571 211. http://irmm.jrc.ec.europa.eu
Telephone: direct line (32-14) 571 229. Fax: (32-14) 571 783.

E-mail: jrc-irmm-crl-mycotox@ec.europa.eu

13.3. Homogeneity test

Material	Analyte	s^2_{sam}	s^2_{an}	σ^2_{all}	N	c
Sample A	DON	805	421	543	10	1450
	ZON	626	336	669	10	1600
	T-2	0.282	10.6	8.84	10	27.3
	HT-2	21.2	131	117	10	354
Sample B	DON	11.8	31.8	45.2	10	117
	ZON	0	1.36	1.02	10	3.29
	T-2	0	1.48	0.188	10	1.85
	HT-2	0.964	1.34	3.14	10	7.26

s^2_{sam} – between-sample variance
s^2_{an} – analytical or within-sample variance
σ^2_{all} – allowable between-sample variance
N – number of units tested
c – critical value, equal to 0.3*target SD for the PT, according to ISO 13528, Annex B

13.4. Acknowledgement of receipt form

EUROPEAN COMMISSION
DIRECTORATE-GENERAL
JOINT RESEARCH CENTRE
Institute for Reference Materials and Measurements
European Union Reference Laboratory for Mycotoxins

EURL
European Union Reference Laboratory
Mycotoxins

Geel, 30th May 2012

PROFICIENCY TESTING MATERIALS RECEIPT FORM

Name:
Institute:
Address:
Member State:

NOTE: STORE ALL MATERIALS IN A FREEZER AT -18 °C!

Please ensure that the items listed below have been received undamaged, and then check the relevant statement:

Date of the receipt	
All items have been received undamaged	YES □ / NO □
If NO, please list damaged items:	

Contents of the parcel

a) 2 test materials for analysis:
- Sample A
- Sample B

b) An envelope with documents:
- A copy of instructions
- Participation code
- Questionnaire

Signature / Stamp:

Please fax or e-mail the completed form to:

Zoltan Kunsagi
JRC-IRMM-FSQ
EURL Mycotoxins
Retieseweg 111
B-2440 Geel, Belgium
Tel: +32-14-571 313
FAX: +32-14-571 783
E-mail: zoltan.kunsagi@ec.europa.eu

13.5. Questionnaire

Milc questionnaire

Comparison for PT 2012 DON, ZON, T-2, HT-2

Please fill in your results and answers to the questions. Print the final pdf and return the signed and stamped copy by fax +32 14 571 783 or by e-mail to JRC-IRMM-CRL-MYCOTOX@ec.europa.eu

Submission Form

1. **How many samples does your laboratory analyse for the following mycotoxins per year?**

Questions/Response table	DON	HT-2	T-2	ZON	Info
a) <50	○	○	○	○	
b) 50-150	○	○	○	○	
c) 151-500	○	○	○	○	
d) 500<	○	○	○	○	

2. **Which food or feed matrices does your laboratory analyse for DON, ZON, T-2 and HT-2 on a routine basis the most? (maximum 3)** *

3. **Are you accredited for the determination of these mycotoxins from cereals?**

Questions/Response table	DON	HT-2	T-2	ZON	Info
Accredited for:	☐	☐	☐	☐	

4. **Proficiency test samples: DON in cereals**

4.1. Please indicate the LOD for DON of the method used (µg/kg). *

4.2. Please indicate the LOQ for DON of the method used (µg/kg). *

5. **Proficiency test samples: ZON in cereals**

5.1. Please indicate the LOD for ZON of the method used (µg/kg). *

5.2. Please indicate the LOQ for ZON of the method used (µg/kg). *

6. **Proficiency test samples: T-2 in cereals**

6.1. Please indicate the LOD for T-2 of the method used (µg/kg). *

6.2. Please indicate the LOQ for T-2 of the method used (µg/kg). *

7. **Proficiency test samples: HT-2 in cereals**

7.1. Please indicate the LOD for HT-2 of the method used (µg/kg). *

7.2. Please indicate the LOQ for HT-2 of the method used (µg/kg). *

8. **What is your main procedure for recovery estimation?** *

- ○ a) Internal Standard to Extract
- ○ b) Internal Standard to Sample
- ○ c) Standard solution to Blank
- ○ d) other

8.1. If other please specify! *

9. **During the analysis did you need to include any over night stop?** *

- ○ a) Yes
- ○ b) No

9.1. If YES, please state for which samples and at what stage of the analysis. *

10. **Please indicate the sample amount (in grams) for extraction!** *

11. **What was the solvent to sample ratio used during extraction (in mL/g)?** *

12. **What was the extraction solvent used?** *

13. **What was the extraction mode (e.g. blending or shaking)?** *

14. **What was the extraction time?** *

15. **What type of clean up methodology was used (e.g. immunoaffinity column)?** *

16. **If you used immunoaffinity columns...**

16.1. ... please specify the manufacturer of the immunoaffinity columns you used during the analysis!

17. **What type of detection method did you use?** *

- ◯ a) HPLC-FLD
- ◯ b) LC-MS/MS
- ◯ c) other

17.1. If HPLC-FLD, please specify your method (type of column, injection volume, mobile phase etc.)! *

17.2. If LC-MS/MS, please specify your method! *

17.3. If other, please specify the type of your method! *

18. **How did you integrate the signals?**

- ◯ Automatic
- ◯ Manual

19. **Did you encounter any problems during the analysis?** *

- ◯ a) Yes
- ◯ b) No

19.1. If YES, what were the specific problems and to which samples do they apply? *

20. **Did you notice any unusual observations which, however, did not seem to have any effect on the results?** *

- ◯ a) Yes
- ◯ b) No

20.1. If YES, what were these observations and to which samples do they apply? *

21. **Did you find the instructions distributed for this PT adequate?** *

- ◯ a) Yes
- ◯ b) No

21.1. If NO, which parts do you think can improve? *

22. **What is your opinion about the registering / reporting format of this interface?**

23. **Any other comments you wish to address?**

13.6. Experimental details

Results and method performance characteristics for DON

Lab Code	Technique	Sample A		Sample B		Coverage factor	Recovery [%]	LOD [µg/kg]	LOQ [µg/kg]
		Result [µg/kg]	Uncertainty [µg/kg]	Result [µg/kg]	Uncertainty [µg/kg]				
101	LC-MS/MS	897	24	279	24	2	70	30	60
102	LC-MS/MS	668	134	295	59	2	95	8	40
103	HPLC	512	127	246	68	2	100	15	50
104	LC-MS/MS	495	82.2	106.8	17.7	2	100.5	8.6	21.5
105	HPLC	800	90	400	43	2	78.8	50	100
106	HPLC	596.5	68.6	448.9	51.6	2	56.8	30	60
107	LC-MS/MS	502.7	90.6	86.5	16.4	2	102	10	30
108	LC-MS/MS	605	182	255	77	2	91	35	64
109	HPLC	792.4	110.53	321.94	44.91	2	66.88	52	156
110	HPLC	507	152	266	80	2	90	30	100
111	HPLC	590.5	89.1	275.1	50.5	2	88.7	20	100
112	HPLC	391.6	117.5	213.6	64.1	2	95	7	25
113	GC-MS	720	266	340	126	2	83	5	10
114	LC-MS/MS	577	173	260	78	2	100	200	200
115	LC-MS/MS	491	181.7	240	90.7	2	109	10	50
116	LC-MS/MS	593.4	322.3	264.4	110.1	2	69.2	30	50
117	HPLC	578	173	268	80.4	2	96	20	50
118	HPLC	522	104	245	50	2	100	30	100
119	LC-MS/MS	795.3	124.8	325.9	59	2	89.2	5	10
120	HPLC	431	12	241	12	2	79	1.1	3.2
121	HPLC	428.3	96.8	268.4	60.7	2	90.9	15	49.5
122	HPLC	651.1	45.6	286.4	20	2	94.75	not determined	50
123	HPLC/DAD	586.2	146.6	252.2	63.1	2	99	25	80
124	HPLC	771.2	115.7	327.5	49.1	2	89	40	120
125	GC-MS	590	250	286	143	2	96	15	50
126	HPLC	642	141	280	62	2	79.5	20	30
127	LC-MS/MS	420	27	348	27	2	94	50	20
128	LC-MS/MS	558.77	56.05	256.67	57.06	2	98.58	7.5	15
129	LC-MS/MS	661	46	297	21	2	107	19	57
130	GC-MS	598	209	229	80	2	91	50	100
131	LC-MS/MS	581.5	145.4	248.5	62.1	2	93.5	44	44
132		*No result*		*No result*					
133	LC-MS/MS	505.5	202.2	205.5	82.2	2	100	10	25
134	LC/MS	450	127.4	179	50.7	2	85.4	35	115.0 and for infants and young children 55.0
135	LC-MS/MS	450	167	266	100	2	109	10	50

Results and method performance characteristics for ZON

Lab Code	Technique	Sample A		Sample B		Coverage factor	Recovery [%]	LOD [µg/kg]	LOQ [µg/kg]
		Result [µg/kg]	Uncertainty [µg/kg]	Result [µg/kg]	Uncertainty [µg/kg]				
101	LC-MS/MS	528	24	23	24	2	97	10	20
102	HPLC	354	70.8	25.7	5.14	2	92	2	10
103	HPLC	579	141	32	12	2	72	3	10
104	LC-MS/MS	406	60.5	<25				12.6	25
105	HPLC	414	35	33	2.84	2	80	1	2
106	HPLC	444.1	55.5	37.5	4.7	2	102.7	6	11
107	LC-MS/MS	482.7	95.5	33.2	6.6	2	110	1	3
108	LC-MS/MS	489	196	23	9	2	97	1	2
109	HPLC	393.93	71	22.13	6.67	2	104.81	10	20
110	HPLC	404	121	29	9	2	90	3	9
111	HPLC	475.5	85.6	20.5	3.7	2	104.2	3	10
112	HPLC	493	123.3	30.2	7.6	2	99	2	5
113	HPLC	<3		39.7	11	2	80	3	6
114	LC-MS/MS	514	226	30	13	2	100	10	10
115	HPLC	481	173.2	30.5	8.2	2	106	0.5	10
116	LC-MS/MS	585	321.2	39.6	11.6	2	86	6	20
117	HPLC	476	143	30.3	9.1	2	91	2	5
118	HPLC	310	62	<25			120	8	25
119	HPLC	456.4	78	29.2	7.6	2	89.4	0.3	3
120	HPLC	504	10	32	10	2	95	0.9	2.7
121	HPLC	433	71.9	26.4	4.4	2	91	10	33
122	HPLC	472.1	23.6	32.1	1.6	2	101.91	not determined	20
123	HPLC/FLD	414.3	103.6	30.9	15.5	2	72	5	15
124	HPLC	481.6	72.2	24.5	3.7	2	96	6.5	20
125	HPLC	414	124.2	29	8.7	2	100	5	15
126	HPLC	493	79	31.5	5	2	98.9	2	4
127	LC-MS/MS	267	3	26.5	3	2	72	5	2
128	HPLC	465.56	49.35	32.82	1.48	2	95	0.22	0.43
129		*No result*		*No result*					
130	HPLC	428	74	28	5	2	79.5	20	50
131	HPLC	461.5	50.8	31.1	3.4	2	93	10	10
132	HPLC	462.2	75	29.5	6.9	2	99.4	5	15
133	LC-MS/MS	349.7	139.9	25	10	2	79	10	25
134	LC/MS	436	152.6	33	11.6	2	98	4.5	15
135	HPLC	471	170	29.9	8	2	106	0.5	10

Results and method performance characteristics for T-2

Lab Code	Technique	Sample A		Sample B		Coverage factor	Recovery [%]	LOD [µg/kg]	LOQ [µg/kg]
		Result [µg/kg]	Uncertainty [µg/kg]	Result [µg/kg]	Uncertainty [µg/kg]				
101	LC-MS/MS	100	24	<20		2	95	10	20
102	LC-MS/MS	59.6	11.9	27.5	5.5	2	85	2	10
103	LC-MS/MS	51	18	27	10	2	97.8	0.1	0.5
104	LC-MS/MS	54.3	9.6	20.7	3.7		64	4.2	9.5
105	ELISA	133		60		2	115.8	20	50
106	LC-MS/MS	66.17	11.2	28.7	4.9	2	104	3	5
107	LC-MS/MS	49	9.8	30.6	6.2	2	123	1	3
108	LC-MS/MS	48	8	22	4	2	67.5	1	3
109	LC-MS/MS	46.66	10.59	34.44	8.51	2	85	10	20
110	LC-MS/MS	57	12	25	7	2	97.4	5	15
111	HPLC	96.2	17.3	46	9.2			1.4	5
112		*No result*		*No result*		2	98		
113	GC-MS	59	19	30	10	2	100	10	20
114	LC-MS/MS	61	13	28	6		100	20	20
115	LC-MS/MS	64	14.1	<50		2	124.9	10	50
116	LC-MS/MS	53.4	3.3	23.6	10.9	2	92	7	22
117	LC-MS/MS	62.6	23.8	31.4	11.9	2	95	3	10
118	LC-MS/MS	27	5.4	11.8	2.5	2	85.3	0.5	1.7
119	LC-MS/MS	48.4	11.7	30.1	7.8	2	69	5	20
120	HPLC	15.2	16	33	16			1.4	4.1
121	LC-MS/MS	80.9	16.2	<20		2	100.0	20	66
122	LC-MS/MS	55.28	8.84	26.08	4.17	2	97	not determined	not determined
123	LC-MS/MS	56.7	14.8	27.6	6.9			2	7
124		*No result*		*No result*		2	99		
125	GC-MS	65	19.5	28	8.4	2	110.7	1.5	4.5
126	LC-MS/MS	40.9	9.1	25.6	5.7	2	107	0.2	1
127	LC-MS/MS	38	14	19.4	14	2	94.79	25	15
128	LC-MS/MS	40.14	11.86	30.55	5.31	2	113	0.18	0.36
129	LC-MS/MS	54	5	24	2.1	2	91	8	24
130	GC-MS	38	11	19	6	2	94	50	100
131	LC-MS/MS	57.8	6.4	25.8	2.8			13	13
132		*No result*		*No result*		2	90		
133	LC-MS/MS	40.5	16.2	18.5	7.4	2	101	10	25
134	GC-MS	53.5	21.7	22.5	9.1		100	1.9	7
135	LC-MS/MS	60	13.3	<50				10	50

Results and method performance characteristics for HT-2

Lab Code	Technique	Sample A		Sample B		Coverage factor	Recovery [%]	LOD [µg/kg]	LOQ [µg/kg]
		Result [µg/kg]	Uncertainty [µg/kg]	Result [µg/kg]	Uncertainty [µg/kg]				
101	LC-MS/MS	143	24	27	24	2	96	10	20
102	LC-MS/MS	196	39	46.7	9.34	2	95	2	10
103	LC-MS/MS	129	39	47	17	2	74	0.1	0.5
104	LC-MS/MS	143.5	34	42.5	10.1	2	89.4	5.8	15.2
105		*No result*		*No result*					
106	LC-MS/MS	173.68	46.9	32.9	8.9	2	89.9	4	7
107	LC-MS/MS	146.4	29.2	40.7	8.2	2	97.4	1	3
108	LC-MS/MS	163	29	45	8	2	125	4	13
109	LC-MS/MS	177	33.39	70.5	13.64	2	50	20	40
110	LC-MS/MS	149	45	34	10	2	100	5	15
111	HPLC	127.3	22.9	19.7	4	2	96.5	1.4	5
112		*No result*		*No result*					
113	GC-MS	140	45	41	13	2	99	10	20
114	LC-MS/MS	166	63	41	16	2	100	20	20
115	LC-MS/MS	162	38.4	<50			103	10	50
116	LC-MS/MS	112.3	49.8	27.7	19.1	2	93.5	8	25
117	LC-MS/MS	163	61.9	43.2	16.4	2	97	10	20
118	LC-MS/MS	159	32	34.9	7	2	92	1.5	5
119	LC-MS/MS	157.9	31.9	51.8	12.3	2	87.3	5	20
120	HPLC	6.8	13	52	13	2	62	1.4	4.3
121		*No result*		<20				20	66
122	LC-MS/MS	170.0	27.2	58.4	9.34	2	100.0	not determined	not determined
123	LC-MS/MS	223	55.8	34.2	8.5	2	91	1	3.5
124		*No result*		*No result*					
125	GC-MS	223	66.9	58	17.4	2	92	1.5	4.5
126	LC-MS/MS	74.1	11.6	30.2	4.7	2	103.6	1.7	2
127	LC-MS/MS	83	53	12.3	53	2	110	100	50
128	LC-MS/MS	65.39	12.21	41.09	6.24	2	84.67	2.16	4.32
129	LC-MS/MS	178	20	48	5.3	2	86	5	15
130	GC-MS	131	39	41	12	2	91	50	100
131	LC-MS/MS	154.5	17	38.5	4.2	2	95.6	12	12
132		*No result*		*No result*				-	-
133	LC-MS/MS	135.3	54.1	31.5	12.6	2	90	10	25
134	GC-MS	177	68.1	36.5	14.1	2	114	2.3	8
135	LC-MS/MS	146	35.6	<50			103	10	50

Reference standards for calibration

Lab Code	Deoxynivalenol	Zearalenone	T-2	HT-2
101	Biopure	Biopure	R-Biopharm Rhone	R-Biopharm Rhone
102	Biopure	Biopure	Biopure	Biopure
103	Sigma	Sigma	Sigma	Sigma
104	Sigma	Sigma	Sigma	Sigma
105	LGC Standards	LGC Standards	R-Biopharm Rhone	
106	Romer	Romer	Romer	Romer
107	Biopure	Biopure	Biopure	Biopure
108	Biopure	Biopure	Biopure	Biopure
109	Trilogy	Trilogy	Trilogy	Trilogy
110	Biopure	Biopure	Biopure	Biopure
111	Sigma	Sigma	Biopure	Biopure
112	Biopure	Biopure		
113	Sigma	Sigma	Sigma	Sigma
114	Biopure	Biopure	Biopure	Biopure
115	Biopure	Sigma	Biopure	Biopure
116	Biopure	Biopure	Biopure	Biopure
117	Biopure	Biopure	Biopure	Biopure
118	Sigma	Sigma	Biopure	Biopure
119	Biopure	Biopure	Biopure	Biopure
120	Trilogy	Trilogy	Trilogy	Trilogy
121	Sigma	Sigma		
122	Biopure	Biopure	Biopure	Biopure
123	Sigma	Biopure	Biopure	Sigma
124	LGC Standards	LGC Standards		
125	Biopure	Biopure	Biopure	Biopure
126	Sigma	Makor Chemicals Ltd	Sigma	Sigma
127	Biopure	Biopure	Biopure	Biopure
128	Sigma	Sigma	Biopure	Biopure
129				
130	R-Biopharm Rhone	Biopure	R-Biopharm Rhone	R-Biopharm Rhone
131	Biopure	Biopure	Biopure	Biopure
132		Sigma		
133				
134	Fluka	Fluka	Sigma	Trilogy
135	Biopure	Sigma	Biopure	Biopure

How many samples does your laboratory analyse for the following mycotoxins per year?

Lab Code	DON	ZON	T-2	HT-2
101	151-500	50-150	50-150	50-150
102	500<	50-150	151-500	151-500
103	50-150	50-150	<50	<50
104	<50	<50	<50	<50
105	151-500	<50	<50	<50
106	<50	<50	50-150	50-150
107	50-150	50-150	<50	<50
108	151-500	151-500	151-500	151-500
109	<50	<50	<50	<50
110	151-500	151-500	<50	<50
111	50-150	50-150	<50	<50
112	<50	<50	<50	<50
113	50-150	<50	50-150	50-150
114	500<	500<	500<	500<
115	50-150	151-500	50-150	50-150
116	50-150	<50	50-150	50-150
117	50-150	50-150	<50	<50
118	50-150	50-150	<50	<50
119	50-150	<50	<50	<50
120	50-150	50-150	<50	<50
121	50-150	50-150	<50	<50
122	50-150	50-150	50-150	50-150
123	50-150	50-150	50-150	50-150
124	<50	<50	<50	<50
125	500<	500<	151-500	151-500
126	50-150	50-150	<50	<50
127	50-150	151-500	151-500	50-150
128	<50	<50	<50	<50
129	50-150	<50	50-150	50-150
130	50-150	50-150	50-150	50-150
131	151-500	151-500	151-500	151-500
132	<50	<50	<50	<50
133	50-150	50-150	50-150	50-150
134	<50	<50	<50	<50
135	50-150	50-150	50-150	151-500

Which food or feed matrices does your laboratory analyse for DON, ZON, T-2 and HT-2 on a routine basis the most? (maximum 3)
Are you accredited for the determination of these mycotoxins from cereals?

Lab Code	Analysed matrices on a routine basis	Accredited			
		DON	ZON	T-2	HT-2
101	wheat and wheat products, maize and maize products	√			
102	corn, wheat, mixed feed	√	√	√	√
103	cereals	√	√	√	√
104	feed for poultry, feed for swine	√	√		
105	Feed-DON,ZON, T2	√	√		
106	cereals products	√	√	√	√
107	flour, wheat, cereal products	√	√		
108	cereals; feed: straw	√	√	√	√
109	cereals	√			
110	cereals, animal feed, breakfast cereals , flour	√			
111	feed, cereal, maize	√	√		
112	maize, barley	√	√		
113	cereals (wheat, barley, oats), feed mixtures	√		√	√
114	varied feed for cattle, pigs and poultry + ingredients	√	√	√	√
115	maize, animal feed, cereals	√	√	√	√
116	mixed feeds	√	√	√	√
117	food: cereals, cereal flour, pasta				
118	flour, cereal, baby food	√	√		
119	flour biscuits snacks breakfast cereals	√	√		
120	flour, cereals, animal feed	√	√	√	√
121	cereals, breakfast cereals, pasta				
122	maize, other cereal products, cereal-based baby foods	√	√		
123	cereals, feed, silage	√	√	√	√
124	maize, wheat, barley	√	√		
125	food and feed based on cereals	√	√	√	√
126	cereals, cereal products	√	√		
127		√	√	√	√
128	cereal based products		√		
129	we are accredited for DON, HT-2 and T-2 in flour and oat.(not ZON)	√	√	√	√
130	feed material, compound feedingstuffs for all species	√	√	√	√
131	feeding stuff, cereals	√	√	√	√
132	not in routine				
133	raw cereals, feed pellets	√	√	√	√
134	cereals, cereals products, feedingstuffs	√	√	√	√
135	maize, animal feed, cereals	√	√	√	√
Accreditation		89%	80%	51%	51%

Please indicate the sample amount (in grams) for extraction!
What was the solvent to sample ratio used during extraction (in mL/g)?
What was the extraction solvent used?
What was the extraction mode (e.g. blending or shaking)?
What was the extraction time?

Lab Code	Sample amount (g)	Solvent to sample ratio	Extraction solvent	Extraction mode	Extraction time?
101	25 g	12.8	MeOH:AcCN:H2O (31:31:38 v/v/v)	blending	3 min
102	15 g	100/15	84:16 ACN:water	shaking	2 hours
103	DON, ZON: 5 g T2-HT2: 10 g	10	acetonitrile:water (75:25) for ZON, water for DON, methanol:water (90:10) for T2-HT2	Ultra-Turrax	2 min for deoxynivalenol and zearalenone 3 min for T2-HT2
104	1	8	ethyl acetate	shaking	30 min
105	DON: 25 g ZON: 25 g T2: 25 g	DON: 8 ZON: 5 T2: 5	DON 200 ml water ZON 125 ml acetonitrile 75% T2 125 ml methanol 90%	blending	5 min
106	DON: 20 g ZON: 25 g T2, HT2: 4 g	4 (DON); 6 (ZON); 3 (T-2, HT-2)	PEG+water (DON); MeOH+water (ZON); ACN+water+acetic ac. (T-2, HT-2)	blending	3 min (DON) 2 min (ZON) 3 min (T-2, HT-2)
107	25 g	8 for DON 5 for ZON 4 for T2 - HT2	water for DON, ACN/water for ZON, MeOH/water for T2 HT2	blending	3 min
108	10 g	5:1	AcN-H2O-HCOOH	shaking	1 h
109	5 g	5 g /40 ml for DON 5 g/25 ml for ZON, T2, HT2	DON: distillated water ZON: acetonitrile 75% T2+HT2: methanol 90%	shaking	20 minutes
110	DON: 25 g ZON: 20 g T2, HT2: 2 g	DON: 40 ZON:7.5 T2 HT2: 8	DON: H2O+PEG ZON: Methanol 75+ H2O 25 T2 HT2: Ethyl Acetate	shaking	30 minutes
111	5 g	For DON - 40/5 ZON - 25/5 T-2, HT-2 - 25/5	For DON - water, ZON - ACN/H2O; T-2, HT-2 - MeOH/H2O	shaking	2 hours
112	6 g	ZON : 5ml/g DON: 10ml/g	ZON - CH3CN:H2O (9:1) , DON - H2O	shaking	ZON:1 hour, DON: 30mins
113	ZON: 20 g DON, HT2, T2: 25 g	3 ml/g (ZON) 4 ml/g (DON, HT-2, T-2)	ACN-water 90:10 (ZON), ACN-water 84:16 (DON, HT-2, T-2)	shaking	60 min (ZON) 120 min (DON, HT2, T2)
114	2.5 g	4	Acetonitrile / Water / Formic Acid = 84/16/1	shaking	2 h
115	DON, T2, HT2: 20 g ZON: 25g	Trics (DON, T2, HT2) 20g/100ml ZON 25g/100ml	Trics ACN:H2O 84:16 ZON ACN:H2O 75:25	blending	5 minutes
116	10 g +/- 0,1 g	4 mL/g	84:16 Acetonitrile/Water	blending	3 min
117	25 g	4 ml/g	DON: water, ZON: Acetonitrile-water, HT-2 and T-2: Methanol-water	shaking	120 min
118	25 g for DON, 5 g for ZON, 2 g for T-2 & HT-2	8 mL/g for DON, 4 mL/g for ZON, 5 mL/g for T-2 & HT-2	water for DON, acetonitrile/water (84/16, v/v) for ZON; acetonitrile/water/acetic acid (79/20/1, v/v/v) for T-2 & HT-2	vortex-mixing and shaking	0.5 h for DON 1 h for ZON 1.5 h for T2 & HT2
119	DON, HT2, T2: 12.5 g ZON: 25 g	8 (DON) 4 (ZEA, HT-2/T-2)	water (DON) ACN/water 75/25% (ZEA) ACN/water 80/20% (HT-2/T-2)	blending	3 min
120	DON: 15 g ZON: 20 g T2, HT2: 20g	DON 8mL/g ZON 7.5mL/g T2+HT2- 5mL/g	DON-H2O, ZON- MEOH/H2O 75/25, T2+HT2- MEOH/H2O 90/10	DON - shaking, ZON – shaking, T2+HT2 - blending	DON- 20m, ZON- 60m, T2+HT2- 2m
121	DON: 20 g ZON: 25 g T2, HT2: 25 g	4	ACN/H2O	blending and shaking	3 min
122	DON: 25 g ZON: 25 g T2, HT2: 2 g	DON 8:1 ZON 5:1 T-2 and HT-2 8:1	DON UPW; ZON 75:25 ACN:UPW; T-2 and HT-2 Ethyl acetate	DON Blender; ZON Blender; T-2 and HT-2 Orbital shaker	DON 2 min ZON 2 min T-2 and HT-2 30 min
123	10 g	10	DON:H2O, ZON:MeOH/H2O, T-2:MeOH/H2O, HT-2:MeOH/H2O	shaking and sonication	60 min
124	DON: 10 g ZON: 20 g	100/10 ml/g DON 50/20 ml/g ZON	H2O DON , CH3CN/H2O 75/25 ZON	blending DON, shaking ZON	3min DON 30min ZON
125	25 g	4	acetonitrile/water 84/16 v/v	stiring	2 hours
126	25 g	4	DON, ZEA: Acetonitrile 84 %. T2. HT2: Acetonitrile: H2O:HAc 79:20:1	shaking	30 min
127	5 g	4	Acetonitrile/water	shaking	30 min
128	ZON: 12.5 g DON: 5 g HT2, T2: 10 g	4	Acetonitrile / water (75:25) for ZON; Acetonitrile / water (84:16) for DON, HT2 aT2	blending for ZON, HT2 and T2; shaking for DON	3 minutes for ZON, HT2 and T2; one hour for DON
129	10 g	40/10	Acetonitril:Water; 84:16	shaking	2 Hours
130	10 g	10	acetonitrile-water	shaking	1 hour
131	DON, T2, HT2:10g ZON: 20g	10 ml/1 g	A Acetonitrile/water 84/16 v/v B (ZON) Acetonitril/water 90/10 v/v	shaking	1 hour
132	20g	150/20	MeOH/Water (75/25)	shaking	1 hour
133	5 g	4	ACN 80%, HAc 1%, water 19%	shaking (overhead)	1 hour
134	25 g	DON- 200/25 ZON - 100/25 T-2/HT-2 - 100/25	for DON: water; for ZON: ACN/water; for T-2/HT-2: MeOH/water	DON,ZON – blending T-2/HT-2 - shaking	DON, ZON - 5 min; T-2/HT-2 - 30 min
135	DON, T2 HT2: 20 g ZON: 25 g	Trics (DON, T2, HT2) 20g/100ml ZON 25g/100ml	Trics ACN:H2O 84:16 ZON ACN:H2O 75:25	blending	5 minutes

What type of clean up methodology was used (e.g. immunoaffinity column)?
If you used immunoaffinity columns please specify the manufacturer of the immunoaffinity columns you used during the analysis!
What is your main procedure for recovery estimation?
During the analysis did you need to include any over night stop?
How did you integrate the signals?

Lab Code	Clean up	If IAC: manufacturer	Recovery estimation	Over night stop	Integration
101	immunoaffinity	R-Biopharm	Standard solution to Blank	No	Automatic
102	mixed bed column		Other: standard addition to sample	No	Manual
103	immunoaffinity column	R-Biopharm	Standard solution to Blank	No	Automatic
104	phase separation		Internal Standard to Extract	No	Automatic
105	Immunoaffinity columns	R-Biopharm Rhone DON YJ388/50, ZON YE 309/50, T2 YC 283/50	Other: CRM DON, CRM ZON , spiked sample for T2	Yes For DON determination, the evaporated samples were analyzed in HPLC the next day.	Manual
106	IAC (DON, ZON)	Vicam: DonTest, ZearalaTest	Other: Standard solution to Sample	No	Automatic
107	immunoaffinity column	R-Biopharm Rhone	Standard solution to Blank	No	Manual
108	MultiSep 226		Standard solution to Blank	No	Automatic
109	DON prep, Easi extract Zearalenone, Easi extract T2 & HT2	R-biopharm Rhone	Standard solution to Blank	No	Automatic
110	immuno-affinity for DON, ZON	R-Biopharm and Neogen	Standard solution to Blank	No	Automatic
111	immunoaffinity column	R-BIOPHARM RÔNE LTD	Standard solution to Blank	Yes For all samples one day extraction and the second day passing through immunoaffinity column	Manual
112	Immunoaffinity Columns	ROMER	Standard solution to Blank	No	Manual
113	IA-column (ZON), MycoSep#227 (DON, HT-2, T-2)	Rhone Diagnostics (ZON)	Standard solution to Blank	No	Automatic
114	no clean up		Standard solution to Blank	Yes All samples, after centrifuging extracts were placed overnight in refrigerator	Manual
115	Trics - MYCOSEP ZON - Immunoaffinity column	R-Biopharm Rhone	Standard solution to Blank	No	Automatic
116	solid phase filtration (Romer 226)		Internal Standard to Extract	No	Manual
117	immunoaffinity column	R-Biopharm Rhone Ltd	Internal Standard to Sample	Yes ZON, HT-2 and T-2: cleaned sample extracts (in methanol) overnight (DON was analyzed immediately)	Manual
118	Immunoaffinity SPE for DON, Immunoaffinity SPE for ZON, Strata-XL SPE for T-2 & HT-2	ROMER	Other: standard addition to sample prior to extraction	Yes For DON the extraction solvent is given to the samples and we let them stay for a night prior to extraction.	Manual
119	IAC (ZEA, DON) Bond Elute mycotoxin (HT-2/T-2)	R-BioPharm	Other: standard solution to sample	No	Automatic
120	immunoaffinity column	DON- R-BIOPHARM DONPREP, ZON-R-BIOPHARM EASI-EXTRACT, T2+HT2-R-BIOPHARM EASI-EXTRACT	Internal Standard to Extract	No	Manual
121	immunoaffinity column	VICAM	Standard solution to Blank	No	Manual
122	Immunoaffinity column in all cases	R-Biopharm Rhone	Other: Spiking of samples	Yes For T-2 and HT-2 (after extraction)	Manual
123	immunoaffinity column	ZON: VICAM, DON: R. Biopharm, T-2 and HT-2: R. Biopharm	Standard solution to Blank	No	Manual
124	immunoaffinity column	R-BIOPHARM DON, VICAM ZON	Standard solution to Blank	No	Manual
125	DON: SPE; ZON: IAC; T2/HT2: SPE + IAC	r-Biopharm	Standard solution to Blank	Yes all samples, after extraction	Manual
126	DON: Immunoaffinity columns, MultiSep Trich ZEA: Immunoaffinity columns. T2, HT2: None	DON: R.Biopharm Rhône, ZEA: VICAM	Standard solution to Blank	Yes Sample preparation one day, LC-analysis 1-2 days after	Automatic
127	MycoSep Columns		Standard solution to Blank	No	Automatic
128	Immunoaffinity ciolumns for ZON; SPE for DON, HT2 and T2	VICAM for ZON; ROMER for DON, HT2 and T2	Standard solution to Blank. Comparison with a reference sample in the case of DON.	No	Automatic
129	Mycosep columns no.225		Standard solution to Blank	No	Automatic
130	Mycosep (DON, T-2, HT-2), immunoaffinity (ZON)	R-Biopharm Rhone	Internal Standard to Sample	No	Automatic
131	MycoSep227Tri ch+ Romer Labs and Easy Extract Zearalenon R Biopharm Rhone LTD	Easy Extract Zearalenon R Biopharm Rhone LTD	Internal Standard to Extract. Standard-addition to feed blank	No	Automatic
132	immunoaffinity column	VICAM	Standard solution to Blank	No	Manual
133	none		Other: standard additions curves (1g sub-samples spiked at 0-10-25-50-500-1000 µg/kg) (plus 13-C IS to extract for matrix effect corrections)	Yes LC-MS/MS analyses performed one day after extractions	Automatic
134	immunoaffinity column	R-Biopharm Rhöne Ltd	Standard solution to Blank	No	Manual
135	Trics - MYCOSEP ZON - Immunoaffinity column	R-Biopharm Rhone	Standard solution to Blank	No	Automatic

Did you encounter any problems during the analysis?
Did you notice any unusual observations which, however, did not seem to have any effect on the results?

Lab Code	Problems	Unusual observations
101	No	No
102	No	No
103	No	No
104	No	No
105	No	No
106	No	No
107	Yes - Gel formation during T2 HT2 extraction with MeOH/H2O. Filtration not possible if not centrifuged first.	No
108	No	No
109	No	No
110	No	No
111	No	No
112	No	No
113	No	No
114	No	Yes - during extraction, sample material sticked to extraction tube
115	No	No
116	No	No
117	No	No
118	No	No
119	No	No
120	No	No
121	No	No
122	No	No
123	Yes - IAC - ZON: unexpected very low recovery	No
124	No	No
125	No	No
126	No	No
127	No	No
128	No	No
129	No	No
130	No	No
131	No	No
132	No	No
133	No	No
134	No	No
135	No	No

Did you find the instructions distributed for this PT adequate?
What is your opinion about the registering / reporting format of this interface?
Any other comments you wish to address?

Lab Code	Instructions	Registering / reporting format	Any other comments
101	Yes	very clear and time saving	The recovery factor value for T2 toxin (29%) is quite low (three replicates), but we decided to report anyway the results for T2 toxin
102	Yes	OK	
103	Yes	satisfied	
104	Yes	O.K.	
105	Yes	good	
106	Yes	lack of button "save and return to main page"	
107	Yes	adequate	
108	Yes	Works well now (after some improvements)	
109	Yes		
110	Yes		
111	Yes	Better than before	
112	Yes	very good	
113	Yes	very feasible	
114	Yes	OK	
115	Yes	OK	
116	Yes	perfect	
117	Yes	Point. 3 Accreditation for DON and ZON is not yet accepted but it was not possible to send this report without any value in 3.	PT-tests are very important for us. This was the first time we analysed HT-2 and T-2 with LC-MS/MS.
118	Yes	Quite good	
119	Yes	too cumbersome	why is there still a need for a signed and a stamped report? who is not trusted? the lab or the https web site or both?
120	Yes		
121	Yes	The question 3 of the questionnaire is not correct. Because if we are not accredited in any of the methods we cannot proceed the validation and submission of the questionnaire. So we had to tick at DON to proceed.	We are not accredited for the DON, ZON, T2, HT-2 methods of analysis
122	Yes	User friendly, no problems encountered	This form was designed with a single multianalyte method in mind!
123	Yes	The reporting format is very clear.	
124	Yes		NO
125	Yes	little bit too less place in point 22.	LCMS/MS: DON=453/213;HT2=168/45;T2=56/29;ZON=492/32; LC:DON=633/319 (sample A/B)
126	Yes	It is not clear how to get further from one page to another.	
127	Yes		
128	Yes	OK	
129	Yes		
130	Yes	Registering format ok, problems with reporting format.	
131	Yes	OK	
132	Yes	Good	The sample was not transported with refrigeration.
133	Yes	straightforward (except absence of 'return' button after filling in results and quest.)	
134	Yes	OK!	Please, send blank sample (same matrix as sample) in the next PT too
135	Yes	OK	

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Author(s): Zoltan Kunsagi, Katrien Bouten, Andreas Breidbach, Carsten Mischke, Stefanka Bratinova, Joerg Stroka

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Abstract

This report presents the results of the ILC of the EU-RL for Mycotoxins which focused on the determination of deoxynivalenol (DON), zearalenone (ZON), T-2 and HT-2 in cereal samples.

Thirty-five participants from 27 countries registered for the exercise. 34 (Sample A) & 34 (Sample B) sets of results were reported for DON, 33 & 32 for ZON, 32 & 28 for T-2 and 30 & 28 for HT-2.

Only z-scores for DON and ZON were calculated and used for benchmarking and in total about 95 % of the attributed z scores were below an absolute value of two for these two mycotoxins, which indicated that most of the participants performed satisfactory or better.

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